Anthrax

Disease Monograph Series – 16

Bacteria | *Bacillus anthracis* | Livestock | Wild Animals

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Table of Contents

Acronyms

AICT	Anthrax immunochromatographic test
AU	African Union
AU-IBAR	African Union Inter-African Bureau for Animal Resources
AU-PANVAC	African Union – Pan African Vaccine Centre
CVO	Chief Veterinary Officer
DG	Director General
DoI	Duration of immunity
DVS	Director Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
IM	Intramuscular
IN	Intranasal
LF	Lethal Factor
mPA	mutant form of Protective Antigen (PA)
NGO	Non-governmental organization
OF	Oedema Factor (also known as Edema Factor)
OIE	World Animal Health Organization
PA	Protective Antigen

PCR	Polymerase chain reaction
SC	Subcutaneous
SHF	Small holder farmer
TPP	Target Product Profile
UEMOA	West African Economic and Monetary Union (Union Économique et Monetaire Ouest Africaine)
WHO	World Health Organization of the United Nations
WP	Work Package
wtrPA	Wild type recombinant Protective Antigen (PA)

Executive Summary

Disease, etiology, epidemiology and impact

Anthrax is a peracute, acute or subacute, highly contagious disease of domestic and wild animals and humans, caused by the bacterium *Bacillus anthracis*. Between outbreaks, the anthrax bacterium survives in the environment as a highly resistant spore. The disease most commonly occurs in herbivores, which are infected after ingesting spores. It causes serious damage to domesticated animals and economic losses. It is also important in wildlife, as it is part of the ecosystem in many natural parks, and poses a threat for endangered species, humans and neighboring livestock. In humans, the disease is acquired from contact with infected animals or animal products.

Anthrax toxin and the capsule are the 2 major virulence factors. The capsule is anti-phagocitary, and the toxin is composed by 3 nontoxic proteins, Lethal Factor (LF), Edema Factor (EF) and Protective Antigen (PA). The PA is the principal antigen responsible for the production of toxin-neutralizing antibodies. Fully virulent *B. anthracis* have two plasmids: pX01, which codes for the protein exotoxin complex and pX02 which encodes the capsule genes.

Transmission of anthrax relies on ingestion, percutaneous/parenteral inoculation, or inhalation of spores. Ingestion of spores is generally associated with drinking from a contaminated water source, or ingesting contaminated grazing, browse, flesh or bones. Animals incubating the disease are not infectious for other animals until they die and bloody discharges from the orifices contaminate the environment. Anthrax spores survive best in alkaline soils that are rich in calcium and have a relatively high moisture and organic content. Spores can survive for years in the environment. The general pattern of anthrax in endemic regions is sporadic cases on an irregular basis, but with some form of seasonal pattern.

Genetic variation: In West Africa, a unique lineage of *B. anthracis* deficient in the surface monosaccharide anthrose, has been identified in Chad, Cameroon, Mali and Nigeria. Anthrose is known to play an immunodominant role in the anthrose-containing locally produced vaccines. It would seem that this variant is a vaccine escape mutant.

Confounding factor: *Bacillus cereus* is a close relative of *B. anthracis.* It is known mainly as a food poisoning bacteria. *Bacillus anthracis*-associated plasmids have been noted among *B. cereus* isolates, and they might be implicated in causing anthrax like disease in animals and humans.

Incidence / Prevalence

Anthrax is endemic in many countries. Thanks to successful national programs, there has been a progressive global reduction of the disease in livestock. Anthrax remains common in tropical Africa, the Middle East,

neighboring countries of the former Soviet Union, parts of Central and Southern America, and parts of Asia. The number of outbreaks is acknowledged as under reported in many countries, and due to the nature of the disease (peracute/acute) there is very limited prevalence data. The WHO estimates between 20,000-100,000 human cases per year.

Classical diagnostic is based on the identification of the agent in smears. There are some challenges in obtaining the traditional methylene blue stain, so adapted formulations have been proposed. It might also require expert skills to identify the agent. Interestingly, a very promising lateral flow device has been developed by the Biological Defense Research Directorate, Naval Medical Research Centre (USNMRC), USA. The test has been validated by AgriBio in Australia, which has been using it for several years in the field. Unfortunately, the USNMRC has no interest in livestock, and AgriBio is not in a position to commercialize as the IP belongs to USNMRC. This seems an opportunity to follow up on diagnostics.

Control

Anthrax is usually a notifiable disease, therefore control procedures are usually prescribed and enforced by national veterinary services. Anthrax control measures are aimed at breaking the cycle of infection and consist basically of a surveillance system, prophylactic procedures, and disease regulatory actions.

B. anthracis is susceptible to most antibiotics; however, because of the peracute nature of anthrax disease in herbivores, by the time the animal shows clinical signs it is usually too late to treat with antibiotics. To treat any animal suspected of incubating the disease, the WHO/OIE/FAO recommends the use of penicillin with streptomycin. A few countries, do not permit antibiotic treatment, and require instead slaughter with appropriate carcass disposal. With the development of the Sterne spore vaccine, a sharp decline in anthrax outbreaks in livestock occurred during the 1930-1980 era. However, a resurgence of this disease in livestock has been reported in some regions, where complacency, a false sense of security or other causes have hindered vaccination.

Current vaccines

Most anthrax vaccines in use in the world at present, utilize the toxigenic, non-capsulating *B. anthracis* 34F2 isolated in 1937 by Max Sterne in South Africa. This variant has lost the pX02 plasmid (pX01+, pX02-), and it is safe for animals and humans. It is used essentially as originally formulated, suspended in saponin and glycerol-saline. The basis of protection is the development of antibodies to the Protective Antigen.

There are also other livestock vaccines used in specific countries or regions. The anthrax strain 1190R is used in Romania, and the anthrax strain 55 is used mainly in Russia, Central and Eastern Europe. Both strains are very

similar to the Sterne stain (pX01+, pX02-). The original Pasteur vaccines which are pX01- (Type 1 and Type 2) have been largely replaced by the Sterne vaccine. The Pasteur Type 1 was used in Italy until 2006. Type 2 is still used in China, but it is unclear to what extent. Italy used to produce the Carbosap vaccine, produced from a pX01+, pX02+ strain, but has stopped now. China also uses the Sterne strain to produce a PA oil emulsion vaccine. There are some references about a vaccine K79-Z used in Ukraine, but information is very limited.

Anthrax vaccines are used alone or in combination. Sterne strain can be combined with Blackleg disease (*Clostridium chauvoei)* and/or with Botulism (*Clostridium botulinum* types C and D).

The Sterne vaccine is cheap, and has been very effective in controlling the disease. It is produced in many laboratories worldwide, but quality issues have been encountered. Promoting the production of good quality Sterne vaccine (by for example training of African manufacturers at AU-PANVAC), and ensuring the Sterne strain they are using is still appropriate, would be of help.

Despite being a good vaccine, there are important issues that hinder the efficacy of the Sterne vaccine. It is affected by the use of antibiotics before and/or after vaccination (which has implications for control measures in the face of an outbreak), immunity takes 7-10 days to develop and even longer in horses, precautions might need to be taken with goats, and adverse reactions have been notified in miniature horses. There is a need for a vaccine that: 1- is not affected by antibiotic treatment, has a stronger initial immunity and can be used at the onset of an outbreak, 2- can be given orally (especially for wildlife), 3- it is safer for humans, 4- does not have withdrawal period, and 5- has a longer duration of immunity (especially desirable for wildlife).

Potential new vaccines

The majority of the work on new vaccines has been focused on human vaccines, and there is only very limited information on new livestock vaccines. Recombinant protein vaccines have the advantage that they potentially would help to overcome the issue of concomitant antibiotic treatment.

Fasanella in 2008 published work on a recombinant PA (rPA) and a trivalent vaccine (TV). Both vaccines produced a quick immune response that persisted at high titres until day 174. Research was discontinued due to the cost of the vaccine.

More recently, the group led by Dr Beyer at the Universität Hohenheim, have been working on evaluating a non-living vaccine based on the protective antigen (PA83), the spore surface glycoprotein (BclA), a capsule conjugate, formalin-inactivated spores (FIS) and a lipopeptide adjuvant (Pam3Cys) in various combinations. They have also developed and evaluated DNA-vaccines. In experiments with goats, the recombinant protein based vaccines showed up to 80% protection from a lethal challenge if formalin-inactivated spores (FIS) were part of the vaccine. In contrast, the DNA-vaccines showed little to no immunogenicity in goats. These recombinant candidates look promising, but further development and evaluation (including stability, as long term stability issues have been identified with rPA vaccines evaluated for humans) is needed. It is important to keep in mind,

that these vaccines will be mainly used for outbreaks, as due to their cost, livestock users wanting to prevent the disease, would likely continue to use the cheaper Sterne vaccine.

A less expensive option than recombinant vaccines, would be the acellular vaccines produced by using the toxins as an antigen in an oil emulsion, as currently being used in China, and being researched by Dr Fasanella in Italy.

One important issue that needs to be clarified in West Africa, is the efficacy of the traditional Sterne vaccine against the anthrose-deficient strains, as that might help explain some of the vaccine failures observed, that have been linked to quality issues (hence might or might not been present also). A trial evaluating the efficacy could be set up with some of the groups working on this subject (and could also include the subunit vaccines produced with Sterne toxins).

Clinical disease overview

Anthrax is a peracute, acute or subacute, highly contagious disease of domestic and wild animals and humans, caused by the bacterium *Bacillus anthracis*. Anthrax appears to be the first disease of humans and animals that was shown to be caused by a micro-organism. Anthrax was also the first disease against which avirulent strains were used as immunizing agents in vaccines. It is also one of the few pathogens that must kill its host in order to propagate.

B. anthracis is also known for its use as an agent in biological warfare during and after the II World War, and subsequent threats during the Gulf war, and bioterrorism. It was calculated that the economic impact of such an act in the USA could amount to $26.2 billion per 100,000 persons exposed.

Anthrax retains a continued place in the ecology of free-ranging wildlife in several regions of the world.

Etiology & Epidemiology

Bacillus anthracis is a bacterium belonging to the family *Bacillaceae.* The genus *Bacillus* includes many species, which are all aerobic and spore forming. *Bacillus anthracis* appears to be a relatively monomorphic species. Recent progress using molecular techniques has enabled a broad separation of isolates into 2 major clonal groups, referred to as A and B. The A and B2 branch isolates are distributed worldwide, while the B1 branch is found only in Southern Africa [1]. It has also been shown that genomics has a strong impact on strain distribution, and this includes the possibility of niche specialisation within a sublineage (see *B. anthracis* lineages).

Actively growing, *Bacillus anthracis* are typically rod-shaped, and in stained smears of blood or tissue fluid obtained from infected animals, the organisms appear truncated, commonly singly or in short chains, and surrounded by a well-developed capsule. Capsules are not formed in culture unless special conditions for their development are provided. *In vitro*, *B. anthracis* grows in long, undulant chains composed of many individuals, which resemble a bamboo pole. Between outbreaks, the anthrax bacterium survives in the environment as a highly resistant spore.

B. anthracis is not a highly invasive organism. The 50% lethal dose for anthrax challenge is much higher by the oral route than by the parenteral route. A major factor determining virulence is the number of plasmids present. Fully virulent *B. anthracis* have two plasmids: pX01, which codes for the protein exotoxin complex and pX02 which encodes the capsule genes. The vegetative anthrax bacilli produce a lethal combination of exotoxins, responsible for the severe clinical signs and post-mortem lesions. The toxin complex consists of 2 separate protein toxins, designated *oedema factor* (OF) and *lethal factor* (LF), and a cell receptor-binding protein called *protective antigen* (PA). Protective antigen combines with OF or LF to form *oedema toxin* (OT) and *lethal toxin* (LT) respectively. The toxin complex acts to reduce phagocytosis, increase capillary permeability and compromise blood clotting mechanism. The net effect is massive oedema (including in the lungs and brain), haemorrhage, renal failure and terminal hypoxia. The different mammals have different sensitivity to these toxins.

The bacterium first multiplies at the site of infection. Then enters the bloodstream, where it is concentrated by the spleen and the other lymphoid tissues until a point is reached when there is a sudden release of organisms and toxins back into the bloodstream, which leads to rapid death in ruminants.

Transmission

Transmission of anthrax relies on ingestion, percutaneous/parenteral inoculation, or inhalation of spores. Ingestion of spores is generally associated with drinking from a contaminated water source, or ingesting contaminated grazing, browse, flesh or bones. Animals incubating the disease are not infectious for other animals until they die and bloody discharges from the orifices spill infection into the environment. The curiosity of cohort animals, which causes sniffing, licking and grazing infected sites, leads to other animals being infected.

Since *B. anthracis* is apparently non-invasive, it is believed that a lesion is necessary for the initiation of infection. In view of associations between higher incidence and dry hot conditions, theories have arisen that at such times the animal is forced to graze dry spiky grass close to the soil, which produces orogastrointestinal lesions. But this is not totally consistent with some epidemiological observations.

Ingestion is probably the most common route of infection in animals. In predators and pigs, oedematous lesions generally develop in the oral and pharyngeal area, while in domestic and wild herbivores, necro-haemorrhagic lesions develop in Peyer's patches or regions of the small intestine, eventually progressing to septicaemia. Osteophagia by pregnant or lactating animals, or animals on rangeland with phosphate deficient soils, is an important cause of infection in certain regions.

B. anthracis may penetrate broken skin or mucous membranes, and this route of infection is most commonly seen in humans who have handled anthrax infected animal products. The animal equivalent is the infection of subcutaneous tissues, generally as a result of mechanical transmission by contaminated biting insects. Cellulitis characterised by subcutaneous swelling is particularly common in horses and pigs. In carnivores, the massive

facial and oral oedema and necrosis are thought to be due to penetration of oral or pharyngeal mucous membranes by bones.

Inhalation is probably the least common route of infection in livestock and wildlife living in the open air, as anthrax spores tend to clump together with surrounding organic material and are not easily aerosolised.

During an anthrax outbreak, each successive victim may become an additional source of infection. The modes of transmission from the infected carcass or bacteraemic animals to the surrounding population at risk will vary in the different environments with their associated species. Anthrax spores may directly contaminate pasture in close proximity to a carcass, and terminally ill animals that are febrile and thirsty may die close to water. The bacilli and spores from a carcass may also be dispersed by water run-off, scavenging birds and carnivorous mammals. Vegetative anthrax do not survive the digestive processes of carnivorous birds and mammals, but the spores pass through the gastrointestinal tract to indirectly contaminate distant sites. In South Africa, browse contamination occurs through non-biting necrophagic flies which fed on the body fluids and proteins of infected carcasses, and then set on nearby vegetation depositing infectious vomit and faecal droplets. Necrophagic flies are “case multipliers”, while in certain regions, haemophagic biting flies have been implicated as important mechanical transmitters in herbivores, acting as “space multipliers” by causing local clustering and centrifugal spatial spread of infection.

Anthrax spores survive best in alkaline soils that are rich in calcium and have a relatively high moisture and organic content. Spores can survive for years in the environment and, under optimal conditions, some may survive for decades or even centuries [1]. Spores can also survive for two years in water, 10 years in milk and up to 71 years on silk threads.

The general pattern of anthrax in endemic regions is that of sporadic cases on irregular basis, but with some form of seasonal pattern. These sporadic events are intermixed with periodic outbreak clusters, or even propagating epidemics that are generally linked to increased densities or concentrations of host species, or abundance of arthropod vectors. The typical “Anthrax seasons” are characterized by hot-dry weather which stresses animals and reduces their innate resistance to infection allowing low doses of spores to be infective [2]. During outbreaks in winter, the infection by ingestion is the most common route, while summer outbreaks are generally associated with peaking populations of flying insects and percutaneous transmission.

Despite being a well-known disease, there are still epidemiological questions that are not answered. The reason why large outbreaks usually affect only one species in the affected area, while the incidence in others, equally susceptible species in the vicinity remains sporadic is not known. But it might be explained by the different ecological niches of the various species. Susceptibility data based on experimental results do not correlate well with the deaths under natural circumstances. Cattle are quite resistant to experimental infection, but are very prone to infection in nature. In contrast, sheep are quite susceptible to experimental infection but may not be so readily infected in nature. Goats, despite being even more susceptible than sheep to experimental infection, account for even fewer cases than sheep. In the wild, there is an apparent preference for a particular species in any one region. Zebras for example, are the most commonly affected in the Etosha National Park (Namibia), with kudu only occasionally affected. In the Kruger National Park (South Africa), kudu is

the principal host, accounting for over 50% of all cases. It would seem that grazing, browsing and flies are the main variables in the different transmission equations. Other important aspects are how plants are eaten: bovines pull plants out of the ground, ingesting a lot of soil, while in contrast, sheep and horses bite plants at ground level, taking relatively little soil.

B. anthracis lineages

Epidemiological or forensic investigations of anthrax benefit from the ability to subtype and geo-position the pathogen. Effective sub-typing systems for the pathogen exist, including systems based on single nucleotide polymorphisms (SNPs), variable number tandem repeats (VNTRs) within a multiple locus VNTR analysis (MLVA), and single nucleotide repeat (SNRs). In particular, VNTR genotyping has described the diversity of the pathogen in numerous countries.

Lista et al. [3] subtyped strains from Cameroon using a 25-marker MLVA, assigning them to a unique "E" lineage. A later study examined the molecular diversity of *B. anthracis* in Chad using VNTRs and concluded the strains represented a novel and phylogenetically distinct A lineage, termed Aβ [4]. Most recently, these same authors examined bovine strains from seven sites in Cameroon and determined they were highly genetically similar to Chadian strains and were also assigned to the Aβ branch [5]. In 2015, it has been published by Blackburn et al [6], that MLVA-25 subtyping of Nigerian, Chadian and Cameroonian isolates indicates that they are closely related genetically, providing evidence that the lineage is widespread in cattle in this West African region.

Relevance to vaccine protection

The surfaces of *B. anthracis* expose a pentasaccharide containing an unusual terminal monosaccharide called anthrose, which has been considered for use as a vaccine (or target for diagnostics). Surprisingly, none of the isolates from Chad, Cameroon and Mali evaluated by Tamborrini [7] were recognized by the MAb specific for anthrose oligosaccharides, suggesting that these West African isolates from the same lineage, are unable to produce anthrose. Immunization of cattle in Chad with a locally produced anthrax vaccine based on anthrose-positive spores of the Sterne strain, elicited an anti-carbohydrate IgG response specific for a synthetic anthrose-containing tetrasaccharide. Further studies suggested an immunodominant role of the anthrose-containing carbohydrate in cattle. The geographic correlation of massive vaccination with the live vaccine strain Sterne (See Section 6), which produces an antigenic response to anthrose, with the lack of anthrose positive *B. anthracis* field strains specifically in this area, leads to the hypothesis that the latter strains represent escape mutants. This hypothesis could be tested by comparing the protective efficacy of a Sterne vaccine and vaccine derived from anthrose deficient *B. anthracis* strain towards anthrose-producing and anthrose-deficient strains. Blackburn [6], who tested Nigerian isolates dating 60 years ago in Nigeria, suggests that this escape mutation was present at least 60 years ago, a timeframe consistent with the mass introduction of the Sterne vaccine in the continent.

Bacillus cereus and its implication in anthrax-like disease

Bacillus cereus is a close relative of *B. anthracis.* It is known mainly as a food poisoning bacteria, able to cause diarrhea and vomiting, but also more severe infections. *Bacillus anthracis*-like plasmids have been noted among *B. cereus*, and they might be implicated in causing anthrax like disease. Bacillus isolates causing anthrax in chimpanzees in Ivory Coast, have been found to have chromosomal properties of *B. cereus* combined with *B. anthracis* virulence plasmids [8]. Also human cases of anthrax like disease have been associated to the presence of *B. anthracis* plasmids in *B. cereus* [9].

In a recent study by Kaminska [10], isolates of B. cereus from Argentina, Kazakhstan, Kenya and Poland were investigated. The plasmids were found in about 17% of the isolates, but their frequencies and expression of replicons differed within and between populations.

It is not clear if the current Sterne 34F2 vaccine would protect animals from this anthrax-like disease.

Clinical Signs

In most species anthrax is characterized by the development of a rapidly fatal septicaemia, resulting in sudden death. The principal lesions are widespread oedema, haemorrhage and necrosis.

Animals

The incubation period under natural conditions, probably ranges from 1 to 14 days. Most animals develop a fever but its severity is extremely variable. It appears to be characteristic for a species and may depend on the number of infecting organisms. The fever usually declines before death.

Ruminants and hindgut digesting herbivores are the most susceptible. Carnivores and primates (including humans) are more resistant to infection, and ostriches the only avian species in which natural infection has been regularly reported. Carcass scavengers are usually highly resistant. Although predators are in general less susceptible to infection by *B. anthracis*, they are highly vulnerable to massive exposure when they feed on infected carcasses, ingesting billions of organisms. Clinical cases in predators are generally more common during the early phases of an epidemic in a new area. Then, they seem to develop strong immunity.

Innate resistance to anthrax appears to depend on the inhibition of the initial germination of spores and/or multiplication of the bacteria. Carnivores, rats and chickens appear to have a high resistance to infection, but once infected, are highly susceptible to the effects of the toxins – as infection progresses, they develop a low-level bacteraemia in the terminal septicaemia. On the other hand, animals such as herbivores, mice, guinea-pigs and rabbits, have a much lower resistance to infection and a relatively high resistance to the toxins, so they develop a high-level bacteraemia in the terminal septicaemia.

Three different manifestations are recognized: peracute, acute and subacute to chronic forms. Cattle, sheep, goats and some wild ruminants, such as kudu, mainly manifest the peracute and acute forms. Horses, donkeys and zebra suffer the acute form. Omnivores (e.g. pigs), carnivores and immunized animals, usually present the subacute to chronic form.

- Peracute form: The course of the disease is usually less than 2 hours. The majority of the animals are found dead without having shown any signs of illness. If there are clinical signs, they include pyrexia, restlessness or anxiety, muscle tremors, dyspnoea, congestion, ruminal stasis, collapse and terminal convulsions. Blood-stained fluid sometimes exudes from the nostrils, mouth and anus.
- Acute form: The course of the disease is usually less than 72 hours. The animals remain standing with their heads hanging and their eyes staring, they are depressed, lag behind the others, walk about listlessly, and lie down frequently. Respiration is laboured and rapid, and scattered small haemorrhages may occur in the visible mucous membranes and skin. Some animals may develop diarrhoea, which is usually haemorrhagic. In lactating cows, milk production decreases, and the small amount of milk produced is either blood stained or yellow. Pregnant animals may abort. Oedema of the tongue and subcutaneous tissues in the throat and ventral parts of the thorax, abdomen and perineum may occur.
- Subacute to chronic form: The course of the disease usually extends for more than 3 days before recovery or death occurs. The most frequent sign is an oedematous swelling of the face, throat and neck following primary infection of the pharynx, pharyngeal tissues and regional lymph nodes. The swelling in the pharyngeal region may become so extensive, that it interferes with respiration (and may result in asphyxia) and the ingestion of food and water.

Humans

Depending on the route of exposure, natural anthrax infection in man can take 3 forms: cutaneous, gastro-intestinal or most seriously, pneumonic anthrax. Recently, a fourth form, injectional anthrax, due to the intake of contaminated heroin, has been documented [11]. See Zoonotic disease at the end of this Section.

Diagnosis

Post-mortem

Is not recommended, because opening the carcass favours sporulation. Regulations in most countries prohibit the opening and post-mortem examination of animals where anthrax is suspected. There is also a zoonotic risk for the person conducting the examination. Animals that have died suddenly, usually in good condition, where rigor mortis is incomplete or absent, and rapid bloating occurs are suspicious. Blood-stained fluid may exude from one or more body orifices. Petechiae and echymoses are often present. The blood is dark and tarry, and frequently does not clot.

Important samples to collect are blood smears for light microscopy and cotton swabs or filter paper blots of blood for culture. These swabs/blots should be allowed to dry to reduce viable contaminants. Although blood samples and smears are preferable, an ear could be cut off, placed in a plastic bag, and transported appropriately to the laboratory.

OIE recognized tests

Identification of the agent:

- Smears of blood or tissues from fresh anthrax-infected carcasses
- Culture on blood agar plates
- Identity confirmation: gamma phage lysis and penicillin susceptibility.
- Capsule visualisation: Smears from blood from ear veins or peripheral veins, exudate from orifices, and, for horses and pigs, from oedematous fluid or superficial lymph nodes. The smears should be dried, fixed either using heat or by dipping in 95-100% alcohol for about 1 minute and air dried, and then stained with polychrome methylene blue (PBM -MacFaydean's reaction). The capsule stains pink, and the bacillus cell stain dark blue. The capsule is not present on *B. anthracis* grown aerobically on nutrient agar or in nutrient broths, but can be seen when the virulent bacterium is cultured for a few hours in a few millilitres of blood. Visualisation of the encapsulated bacilli, usually in large numbers, in a blood smear with PMB is fully diagnostic.
- Difficulties may arise in the case of pigs and carnivores in which the terminal bacteraemia is frequently not marked, or in animals that receive antibiotics before death.
- Recovery from decomposed carcasses, processed specimens (bone meal, hides) or environmental samples (contaminated soil) is often difficult.

Immunological detection and diagnosis

- *B. anthracis* is antigenically very closely related to *B. cereus*, which is considered a ubiquitous component of the environmental microflora. The only unshared antigens are the anthrax toxin antigens produced during the exponential phase of growth, and the capsule of *B. anthracis.*
- Ascoli test: Antiserum raised in rabbits is sued to produce a precipitin reaction. The test lacks high specificity, and appears to be used only in Eastern Europe.
- ELISAs have been developed for the detection of antibody, particularly to the protective antigen. But these tests are only carried out in specialist research laboratories and their results have to be interpreted with a knowledge of an animal's anthrax vaccine history.
- Immunofluorescence: for capsule observation. Not used for routine diagnosis.

Confirmation of virulence with the polymerase chain reaction

- PCR to confirm the presence of the pX01 and pX02 plasmids. Primers mentioned by the OIE Manual have been used in pure cultures or isolates from animals, humans and environmental samples. They may be unsuitable for direct detection, and alternatives for that are published elsewhere.

A skin hypersensitivity test using Anthracin T is widely used in some countries for the retrospective diagnosis of anthrax in animals and humans.

There are no tests available to determine if animal products are free of contamination with *B. anthracis* spores.

Recent developments:

- The simple polychrome methylene blue (PMB) staining established in 1903 remained accepted as a highly reliable, rapid diagnostic test for anthrax for six decades throughout the world. Improvements in disease control led to anthrax becoming rare in industrialized countries and less frequent in developing countries with the result that quality controlled, commercially produced PMB became hard to obtain by the 1980s. Mixed results with alternative methylene blue-based stains then led to diagnosis failures, confusion among practitioners and mistrust of this procedure as a reliable test for anthrax. The traditional PMB preparation as described in the OIE Manual, should be allowed to stand exposed to the air, with occasional shaking for at least one year to oxidise and mature. Owen et al in 2013 [12] published that for laboratories needing a reliable M'Fadyean stain at short notice, the best approach is to have available commercially pure azure B ready to constitute into a solution of 0.03 g azure B in 3 ml of 95% ethanol or methanol to which is then added 10 ml of 0.01% KOH (0.23% final azure B concentration) and which can then be used immediately and through to the end of the tests. Stored in the dark at room temperature, the shelf life is at least 12 months. Smears should be fixed with ethanol or methanol (95-100%), not by heat, and the stain left for 5 min before washing off for optimum effect.

- A lateral flow device has been developed the Biological Defence Research Directorate, Naval Medical Research Centre, USA and AgriBio in Australia [13]. The anthrax immunochromatographic test (AICT) detects PA present within the blood of an animal that has died from anthrax, and provides results in 15 minutes. The diagnostic specificity of the test was estimated to be 100% (99.4–100%; 95% CI) and the diagnostic sensitivity was estimated to be 93.1% (83.3–98.1%; 95% CI). Please see additional information on Section 7.

Most commonly used in low & middle income countries:

- National laboratory: smears and cultures.

Main needs for diagnostics:

a) A diagnostic test that could be used at the point of care by Primary Animal Health Care (PAHC) providers.

b) Tests that can determine if animal products are contaminated with anthrax

Differential diagnosis:

Anthrax should be considered in the differential diagnosis of all cases of sudden death in grazing animals, especially when blood-stained exudate is present at the mouth, nose or anus. Anthrax cases are most often misdiagnosed as clostridial diseases such as:

- Blackleg (*Clostridium chauvoei*)
- Black disease (*Clostridium novyi*)
- Malignant oedema (*Clostridium septicum*) and
- Enterotoxaemia (*Clostridium perfringens* type D)

B. anthracis is a member of the *Bacillus cereus* group, which also contains *B. cereus* and *B. thuringiensis*. Plasmids closely related to pX01 and pX02 have recently been found in a few *B. cereus* isolates that caused anthrax-like disease in people, chimpanzees or gorillas.

Zoonotic disease

Anthrax is an important zoonotic disease and the World Health Organization (WHO) estimates that between 20,000 and 100,000 human cases occur globally per year (Dr Sadjinou presentation at FAO/WHO/OIE meeting in Togo, September 2015). Evidence suggests that humans are relatively resistant to anthrax. The clinical classification refers to the route of infection, that is cutaneous, enteric or inhalational (pulmonary). Cutaneous anthrax accounts for 95% of human cases reported. It is an occupational disease, related to butchering or handling meat from infected animals. Suspected transmission of anthrax to humans by biting flies during animal outbreaks has also been recorded. Infections in drug users have resulted from the infection of heroin contaminated with anthrax spores.

Cutaneous anthrax is characterised by a pruritic papule, which develops around vesicles plus regional oedema. The lesion ulcerates to form a painless scar, with a black necrotic centre. Uncomplicated cases will heal with minimal scarring. Antibiotic treatment does not stop or reverse the development of the skin lesion, but prevents bacteraemia and systemic spread. If untreated, 20% of the cases will progress to severe systemic, and potentially fatal infections.

Enteric anthrax is more common in rural areas of the developing world where carcasses are considered a windfall by protein deprived people. The source of infection is probably a large dose of vegetative bacilli in raw or undercooked meat, rather than spores. There are 2 patterns of the disease: oropharyngeal and intestinal. The oropharyngeal infection may occasionally compromise the airway, and systemic sepsis may occur (as with all forms of anthrax). The intestinal form, depending on its severity may resolve, respond to treatment, or progress to bloody diarrhoea, acute abdomen followed by shock and death.

Inhalation anthrax was historically an occupational disease for wool sorters handling the skins or wool of infected sheep. More recently has been described in musicians playing drums covered with skins from anthrax infected carcasses. Initially it presents as an influenza like illness, that progresses to dyspnoea, shock and collapse, followed by sepsis and death.

In recent decades, anthrax has been propagated for biological warfare and bioterrorism.

Incidence and Prevalence in Selected Countries

Global

Anthrax occurs nearly worldwide, and only a few countries have never reported the disease. Thanks to successful national programs, there has been a progressive global reduction of the disease in livestock. It is acknowledged as under reported in many countries, and is still common in tropical Africa, the Middle East and neighboring countries of the former Soviet Union, parts of Central and Southern America, and parts of Asia.

Anthrax is absent or only sporadic in the middle and higher latitudes of Europe and the Russian Federation. But is still common in some countries bordering the Mediterranean (Albania, Greece, southern Italy, Spain and Turkey). In Canada it is present in the Wood Bison National Park, and it is sporadic in Southern Alberta and Saskatchewan. Also in southern Manitoba. In the USA, the disease is confined to a few persistent pockets in North and South Dakota, Minnesota, Nebraska, Nevada and Texas. The true situation in Latin America is uncertain, because the disease is frequently ignored and underreported. There is a lack of diagnostic facilities, and is enzootic in El Salvador, Guatemala and Mexico. It is absent in Belize and all of the Caribbean except for Haiti. The situation in Colombia is not clear. The disease is well reported in Chile, and it is enzootic in Argentina, Bolivia and Peru, and sporadic in western Uruguay and in parts of Brazil.

In South Africa occurs in wildlife in the Kruger National Park, as in the national parks in Botswana, Namibia, Uganda and Tanzania. The efficient control programs of the past in Zimbabwe and Zambia are a matter of the past, and the disease is now hyperendemic, with significant human losses each year. Epidemics occur in Chad and Ethiopia.

Anthrax is a severe problem in southern and eastern India, with a significant human incidence because the disease is poorly controlled. Outbreaks in wildlife also occur. It is absent however from the western state because of the low soil pH. The disease is a continuing problem in western China, but sporadic in the eastern provinces. Thailand, although essentially free, is afflicted by infected animals imported from Myanmar. The disease is endemic in Cambodia, Vietnam and a number of Indonesian islands. Malaysia is free. Sporadic outbreaks have occurred in China, Taiwan, Japan, Philippines and the Republic of Korea. The situation in the Democratic Republic of Korea is unknown.

An increasing number of countries have been free from Anthrax for a considerable time – Cyprus (1969), New Zealand (1954), Sweden (1981), Ireland (1970) and Malta (1974).

Incidence data by country

There are two main sources, OIE and AU-IBAR (which includes only Africa), but data are not always similar.

Additionally, due to the particularities of the anthrax disease (peracute and acute forms, characterized by the development of a rapid fatal septicaemia, resulting in sudden death, and showing a general pattern in endemic regions of sporadic cases), there is no prevalence data, but there is some data on outbreaks and cases that falls under incidence. This information has also been included.

- Sources used were PubMed and internet engine searches in English (and French for fievre charbonneuse when applicable).
- Efforts have been made to include the year of the study, and not the year of the publication. If they are known to be different, the year of publication is included in the reference.
- For grey literature, links have been included when possible.
- Note that not all papers have been read in full. In many cases, only the abstracts have been read. Critical evaluation of the papers for inclusion has not been conducted.

1- Source: OIE.

Data of outbreaks reported to the World Animal Health Organization (OIE) are shown in Tables 1 and 2. Data are not always reliable, as many countries do not seem to report, or to be reporting consistently over time.

http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

Similar information but presented in a different manner can be seen in Annex 1.

Table 1: ASIA - Anthrax outbreaks notified to OIE from the Asian countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Bangladesh	-	-	+	+	+	+	+	+	+	-	-
India	119	113	111	68	60	84	48	32	29	55	-
Indonesia	+	+	+	>2	-	+	+	+	6	-	-
Myanmar	+	7	5	9	10	5	5	4	1	0	-

Nepal	0	0	0	0	0	5	0	0	+	5	0
Vietnam	5	0	3	3	+	+	>3	>1	1	1	0

Number of cases reported to the OIE by disease and by country:
- No information, + Present but quantitative data not known, ? Disease suspected

Table 2: AFRICA - Anthrax outbreaks notified to OIE from the Asian countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso	15	21	11	11	6	19	5	9	5	3	4
Ethiopia	179	2254	755	430	>215	627	340	638	491	145	
Ivory Coast	+	0	4	>2	>2	6	12	+	1	0	0
Kenya	10	53	39	11	31	32	16	10	8	24	6
Madagascar	1	6	>1	+	?	0	0	0	0	0	
Malawi	0	0	0	0	0	0	0	0	-	-	-
Mali	3	2	0	2	1	0	0	1	0	0	2
Mozambique	3	+	0	1	0	?	0	0	0	0	
Rwanda		26	>22	28	?	+	-	-	?	-	-
Senegal	2	0	1	5	1	2	2	3	7	>1	+
South Africa	1	8	8	7	9	205	27	110	38	146	-
Tanzania	14	12	14	+	>4	5	+	>1	+	+	1
Uganda	+	?	0	0	0	2	1	+	+	0	-
Zambia	-	2	4	7	7	6	5	2	2	3	-

Number of cases reported to the OIE by disease and by country:
- No information, + Present but quantitative data not known, ? Disease suspected

The OIE, also includes zoonoses data. The number of human cases and deaths are reported by the countries. Data from the countries of interest, can be seen in the table below.

http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Zoonoses

Table 3: Human cases and deaths due to Anthrax as reported to the OIE.

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Bangladesh						C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: 225
India				C: 210, D: 58			C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +
Indonesia	C: 25	C: 15, D: 1	C: 74, D: 5	C: 20, D: 0		C: 31, D: 1	C: 41, D:0		C: 11, D: 1	
Myanmar				C: 14, D: 0	C: 7, D: 0	C: 7, D: 0	C: 12, D:0	C: 38, D: 0	C: 23, D:0	
Nepal										
Vietnam	C: +, D: +				C: 6, D: 0		C: +, D: +			
Burkina Faso	C: +, D: +	C: +, D: +								C: 1244 D:25
Ethiopia	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +		C: +, D: +		
Ivory Coast		C: +, D: +		C: +, D: +		C: 5, D: 3	C: 5, D: 3			
Kenya			C: 10, D: 0	C: 19, D: 2	C: 9, D: 0	C: 15, D: 0	C: +, D: +	C: +, D: +	C: +, D: +	C: 134, D: 0
Madagascar										
Malawi	C: +, D: +									
Mali				C: +, D: +						
Mozambique										
Rwanda						C: 18, D: 3	C: +, D: +			
Senegal										
South Africa		C: +, D: +	C: +, D: +	C: +, D: +				C: +, D: +		
Tanzania	C: +, D: +		C: +, D: +	C: +, D: +					C: +, D: +	C: 47, D: 7
Uganda										
Zambia							C: 233, D: 6			
C: Cases										
D: Deaths										

2- Source: AU-IBAR.

The African Union Inter-African Bureau for Animal Resources also has a notification system. Data are published in the Pan African Animal Resources Year Books. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart**=). Similarly to the OIE, many countries do not seem to consistently report the outbreaks. Note that the number of outbreaks reported often does not match those reported to the OIE.

Table 4: Human cases and deaths due to Anthrax as reported to the OIE.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso		19		20	6	19	5	9	5	3	
Ethiopia			478	1997	NS	489	452	601	1067	498	
Ivory Coast				3	2	6	12	5			
Kenya		28	18	5	NS		5		2	20	
Madagascar		21	2	0							
Malawi											
Mali				5	4			1			
Mozambique											
Rwanda				29	NS	18	25	19	19		
Senegal		12		15	1	2	1	4	6		
South Africa		19	9	46	3	195		98	46	145	
Tanzania		16	12		NS	NS		1	1	1	
Uganda						2	5	2		2	
Zambia			4	0	5	4	2		2	1	

Regional

ASIA

Bangladesh

The animal anthrax, locally known as *'Torka',* is believed to have been enzootic in Bangladesh for a long time, and historically human outbreaks were always preceded by animal outbreaks.

There is data available on anthrax human cases from the Institute of Epidemiology, Disease Control and Research of Bangladesh (IEDCR). See Fig 1 for the map of cutaneous anthrax in Bangladesh. **http://www.iedcr.org/index.php?option=com_content&view=article&id=66&Itemid=88**

Currently, there is the Bangladesh anthrax project, under the One Health Network South Asia. **http://www.onehealthnetwork.asia/sites/bangladesh-anthrax-project**

Data of anthrax outbreaks in Bangladesh is shown in Table 5 below.

Table 5: Anthrax outbreaks in Bangladesh.

Year	Area	Species	No. of cases	Reference
2014	Various	Humans (cutaneous anthrax)	Shirajganj: 42, Meherpur: 149 Narayanganj: 8, Tangail: 26	IEDCR (see link in note under Bangladesh)
2013	Various	Humans (cutaneous anthrax)	Shirajganj: 23, Tangail: 77 Gopalpur: 37, Meherpur: 187 Chadanga: 40	IEDCR (see link in note under Bangladesh)
2012	Various	Humans (cutaneous anthrax)	Shirajganj: 74, Kushtia: 5 Bogra: 16, Tangail: 14, Meherpur: 67	IEDCR (see link in note under Bangladesh)
2012	Passive surveillance	Buffaloes, cattle and goats	2095	Mondal, 2014 [14]
2011	Passive surveillance	Buffaloes, cattle, goats and sheep.	1668	Mondal, 2014 [14]
2011	Various	Humans (cutaneous anthrax)	Rajshaji: 21, Shirajgang: 65 Meherpur: 53, Tangail: 29	IEDCR (see link in note under Bangladesh)

			Bogra: 40, Pabna: 32 Chapai Nawabganj: 38	
April-Sept 2011	Sirajgani, Pabna and others	Humans	Sirajgani: 61, Pabna: 32 Bogra: 28, Meherpur: 39, Tangail: 14	Siddiqui et al, 2012 [15]
Aug – Oct 2010	Various	Humans (cutaneous anthrax)	Pabna: 69, Sirajganj: 219 Kushtia: 49, Tangail: 26 Meherpur: 82, Manikganj: 8 Shatkhira: 1, Lalmonirhat: 107 Rajshahi: 8, Narayangonj: 12 Laxmipur: 25, Chittagong: 1	IEDCR (see link in note under Bangladesh)
2010	Passive surveillance	Buffaloes, cattle, goats and sheep.	2174	Mondal, 2014 [14]
2010	12 districts	Cattle Humans	Cattle: 104 Humans: 607	Fasanella, 2013 [16]
2009-2010	14 outbreaks	Cattle, goats, sheep Humans	Animals: 140 (cattle 69%, goats 29%, sheep 2%), Humans: 243	Chakraborty et al, 2012 [17]

India

The disease is enzootic in India. During 2014, there were 30 outbreaks reported in cattle (involving 1878 dead animals) and 25 in small ruminants (involving 302 animals). Source: Annual report 2014-2015. Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India. **http://dahd.nic.in/dahd/WriteReadData/Animal%20Husbandry%20English%202014-15%20(1).pdf**

During 1991-2010, anthrax was reported in eighteen states of India *viz*., Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujrat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamil Nadu, and West Bengal. In India, the outbreaks usually affect cattle, followed by sheep/goat, buffalo, pig and elephant. The number of outbreaks decreased in 2007-08 and maintained at approx. 60 outbreaks per year in subsequent years. The cumulative outbreak data of bovines from 2001-2010, shows the higher number of outbreaks in West Bengal (142), with a total of 631 cases

and 564 deaths. The highest number of outbreaks in small ruminants, especially sheep, are recorded in AP and Karnataka, which might reflect the high sheep population in those areas (Source: Vision 2030 - Project Directorate on Animal Disease Monitoring and Surveillance, Karntaka, India. **http://www.icar.org.in/files/Vision%202030_PDADMAS-11-01-2012.pdf**).

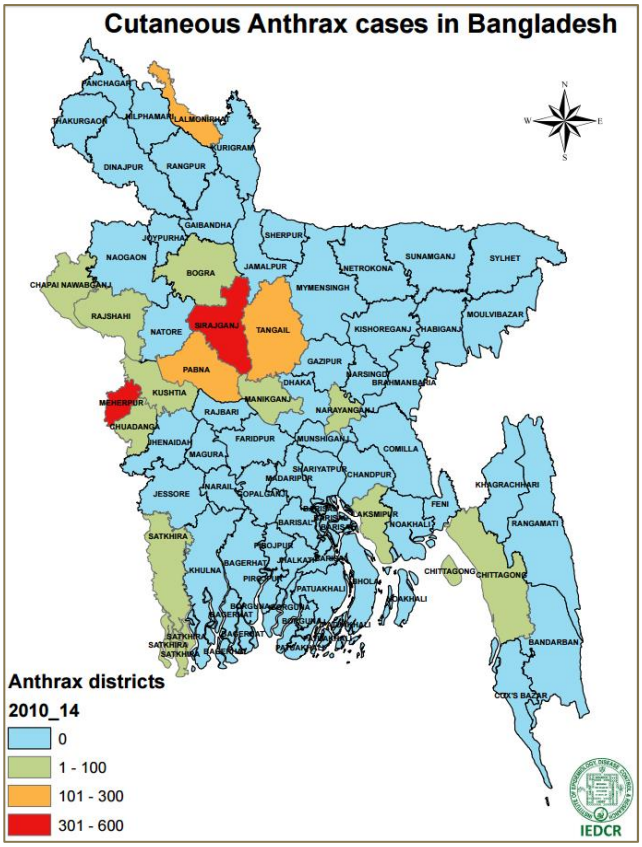


Figure 1: Cutaneous anthrax in Bangladesh 2010 – 2014. (Source: IEDCR. http://www.iedcr.org/pdf/files/anthrax/Anthrax_2010-14_1.pdf**)**

A seasonal fluctuation in the number of anthrax outbreaks has been observed. Most of the anthrax outbreaks are reported in post-monsoon season, from July to September and November to January in different parts of India. Several Southern states such as Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Orissa are common endemic regions with sporadic human anthrax cases reported from time to time. From the Union Territory of Pondicherry, 28 cases of anthrax were detected in 1999 and 2000. Both, animal as well as human anthrax cases are reported usually from certain anthrax endemic districts like Chittoor, Cuddapah, Guntur, Prakasam and Nellore of Andhra Pradesh. In 2006, some cases were noticed near Narsinghpur, Madhya Pradesh also. In 2007, 20 people were affected in two cutaneous anthrax outbreaks in Murshidabad district, West Bengal. These anthrax outbreaks were caused due to slaughtering of sick cattle and subsequently handling of meat without

taking proper preventive measures. An increase in number of animal and human anthrax cases has been observed in this area in recent past. During a tenure of 10 years, anthrax outbreaks were reported at least 61 times from Orissa affecting 750 people. The outbreak of anthrax is a common phenomenon in this area because the tribal population mainly depends on forests for livelihood. Most of the human anthrax cases occur in agricultural workers due to handling of meat or hides of diseased animal. An anthrax outbreak was reported in Orissa, India in 2013 where several people died due to consumption of infected goat meat. Recently, nine cutaneous anthrax cases were reported from the tribal populations of Midnapur, West Bengal in India [18].

Anthrax is also present in wildlife in India, affecting leopards and elephants [19].

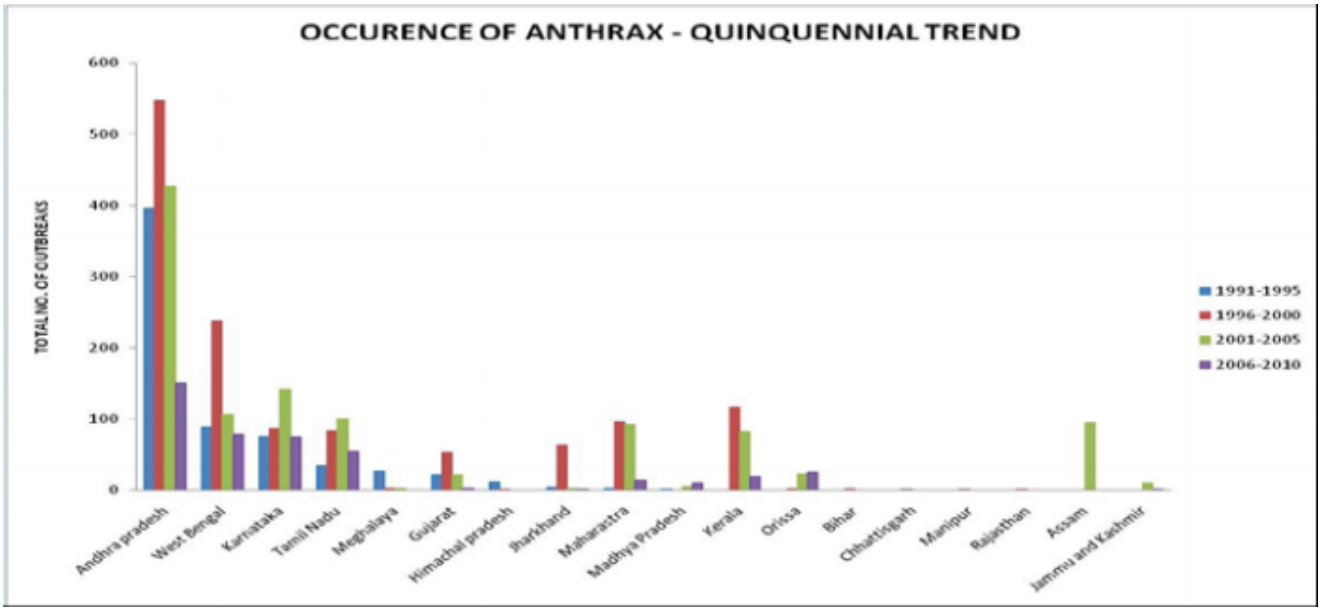


Figure 2: Occurrence of Anthrax in India 1991-2010.
(Source: P.D. ADMAS 2011. http://www.pdadmas.ernet.in/pdf/News%20Letter%20July-Dec%202011.pdf**).**

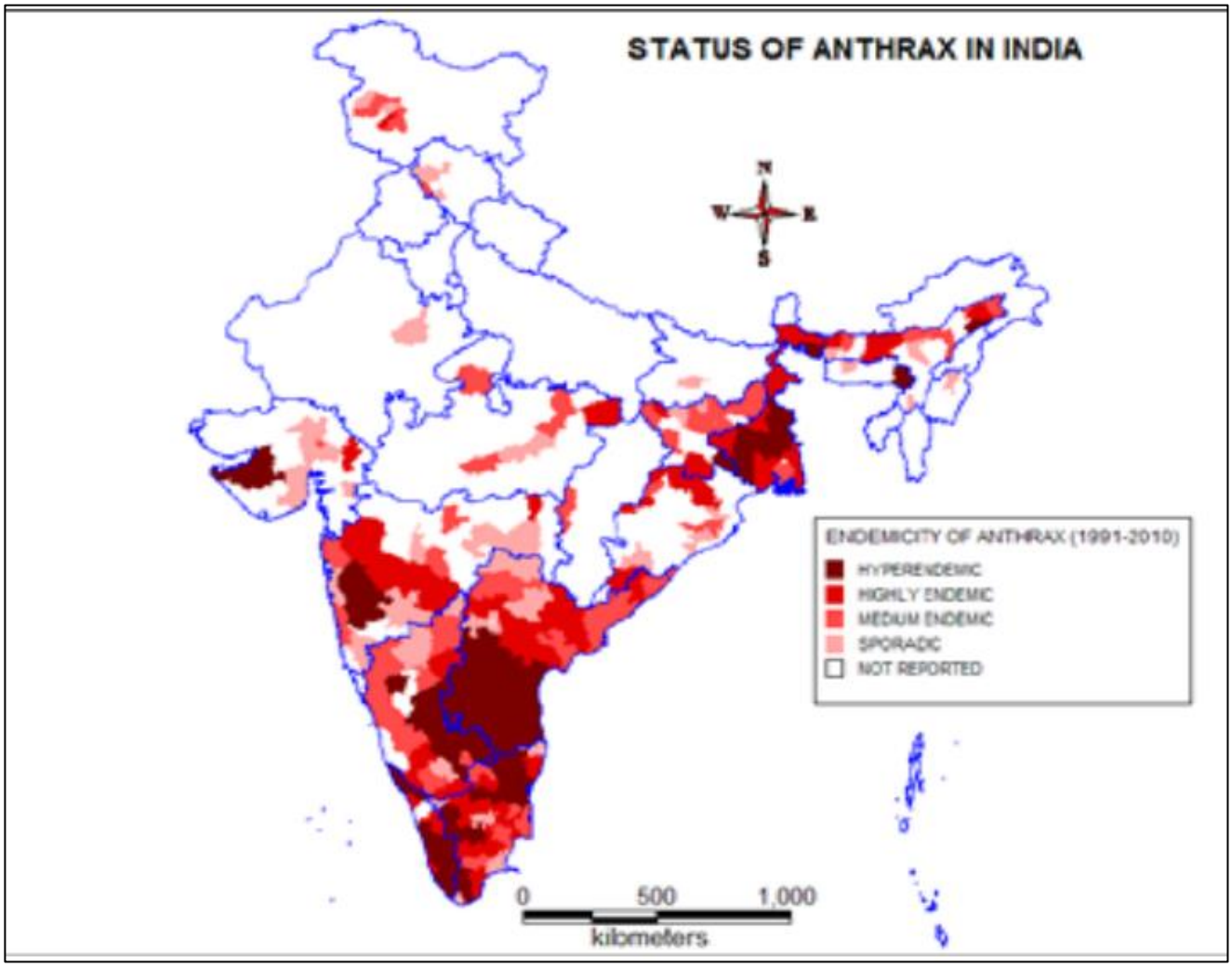


Figure 3: Status of Anthrax in India. (Source: Epidemiology of Anthrax in India, 2012. http://nadres.res.in:8080/Nadres_Uploads/UploadedFiles//VetEpiReports/SecondSlot/Tech%20Anthrax%20final.pdf**).**

Indonesia

No publications found on ProMed.

Information from the Jakarta Post mentions that anthrax is endemic in parts of Indonesia. The disease was first recorded in Indonesia in 1885; Jambi, Bogor and Purwakarta in West Java, Boyolali and Salatiga in Central Java and Sumbawa, West Nusa Tenggara, are endemic areas.
(**http://www.thejakartapost.com/news/2001/10/20/anthrax-endemic-parts-indonesia.html**)

There is also information on the internet, about an outbreak in which more than 100 cattle and buffaloes have died in Laikang and Punaga in Takalar regency (South Sulawesi province).
http://www.globalmeatnews.com/Industry-Markets/Anthrax-detected-in-Indonesia

Myanmar (Burma)

No information available on PubMed or on the internet for incidence or prevalence.

Nepal

No information available on PubMed or on the internet for incidence or prevalence.

V*ietnam*

No information available on PubMed.

2014: A case involving nine people, including adults and children with symptoms of cutaneous anthrax was reported in the northern Ha Giang province, during Sept/Oct 2014. **http://outbreaknewstoday.com/vietnam-province-reports-9-cases-of-cutaneous-anthrax-91036**

2011: The Vietnam Administration of Preventive Medicine notified that the provinces of Lai Chau, Dien Bien and Ha Giang experienced anthrax outbreaks in humans. In Dien Bien Province, the first anthrax case was reported after an ethnic minority resident slaughtered two buffaloes that had died from diseases, and invited other villagers to eat. The unidentified resident also sold the meat of nine sick buffaloes.
http://www.thanhniennews.com/health/mountainous-provinces-in-northern-vietnam-warned-of-anthrax-outbreak-11075.html

AFRICA

Burkina Faso

No recent publications found on PubMed. No information found on the internet in English or French.

Information obtained from the presenation by Dr Estelle Kanyala, Coordinator of the surveillance network of animal diseases (from the Burkinabe DVS office) at the Regional meeting on Anthrax in West Africa organised by FAO in Togo in September 2015. The presentation is not available on line, but was obtained from Dr Bedane of FAO.

Livestock outbreaks:

Year	2010	2011	2012	2013	2014
Outbreaks	21	5	6	5	3

The species affected were bovine (39 outbreaks) and sheep (1 outbreak).

Human outbreaks:

Outbreaks were reported in 2012 in the South West and Hauts Bassins. In 2013 in the South West.

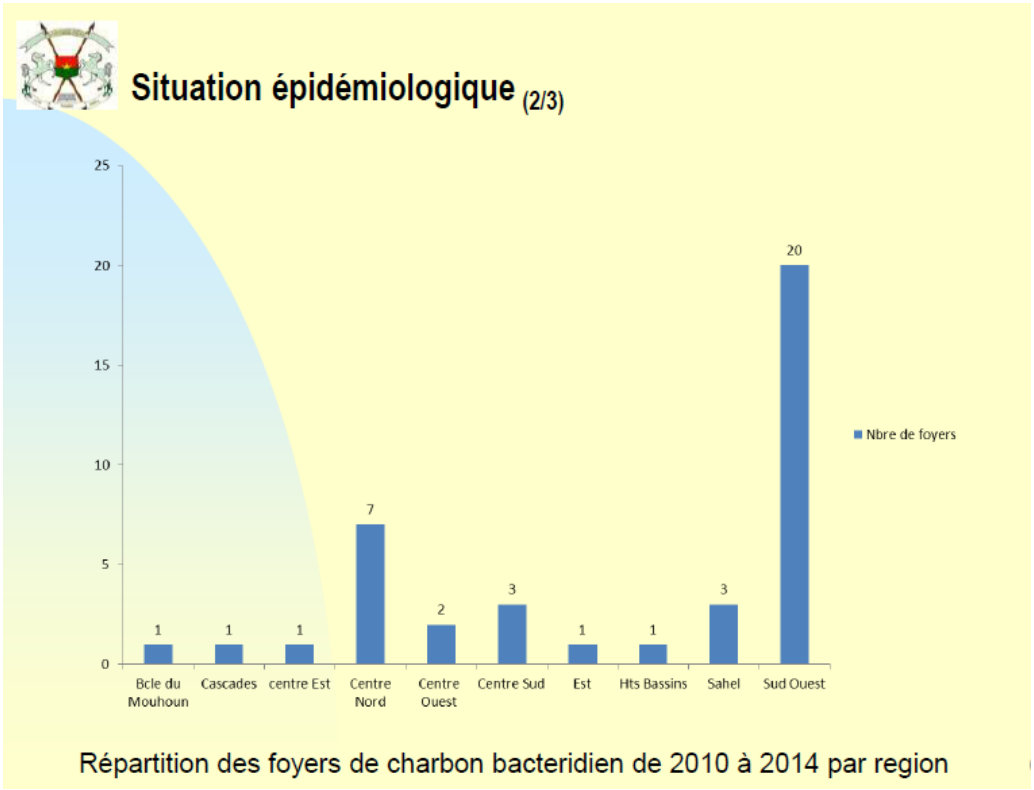


Figure 4: Anthrax human outbreaks in Burkina Faso 2010 – 2014 (Source: Dr Kanyala, Togo 2015)

The presentation makes emphasis on the importance of transhumant movement at national and international level.

Côte d'Ivoire (Ivory Coast)

There is very limited information, and it mainly refers to wildlife.

2001/2: Anthrax was reported in a group of chimpanzees [20] with sudden death from the Taï National Park. Further studies revealed that the *B. anthracis* strain isolated, belonged to a different group than the traditional A and B and it was classified in a new group called F [21].

Ethiopia

Anthrax is endemic in Ethiopia. It occurs in May and June every year ('anthrax season') in several farming localities of the country, causing disease both in humans and livestock. And although suspected cases of anthrax are reported from several districts, few of these are officially confirmed [22].

2012: Report of 3 human cases linked to eating animals that presumably died of anthrax, in West Arsi province (SE Ethiopia). **http://www.revclinesp.es/es/brote-carbunco-una-zona-rural/articulo/S0014256512002792/**

2011-2012: Three patients with periocular anthrax were seen at Jimma University Specialized Hospital, Ethiopia from June 2011 to May 2012 [23]. They were all farmers and had contact with animals.

2002: Case reported in Wabessa village in the Dessie Zuria. The number of infected animals was 1 cow, 8 donkeys and 17 goats and the fatality rate in all cases was 100%, giving mortality rates of 7.7% (cattle), 47.1% (donkeys) and 32.7% (goats). The outbreak also gave rise to three unconfirmed human cases and three unconfirmed deaths. The cutaneous form of anthrax was observed in five of the patients, while three of the patients complained of abdominal pain. The human cases were related to direct contact with infected material and the consumption of infected meat [22]. **http://www.oie.int/doc/ged/D1328.PDF**.

2002: 11 cases were confirmed in Fentale – in the East Shewa area of Oromiya. The anthrax cases were discovered in two of the 18 peasant associations that make up Fentale. The two peasant associations where the bacteria were found – Benti and Kobo – border the Awash National Park.
http://www.irinnews.org/report/32870/ethiopia-anthrax-aggravates-looming-food-crisis-in-east

Kenya

No recent information available on PubMed.

2015: Outbreak at Lake Nakuru National Park reached over 300 dead buffaloes. Other animals affected included rhinos, giraffes, elands, impalas, warthogs and Thomson gazelles. **http://outbreaknewstoday.com/kenya-anthrax-update-300-buffaloes-dead-at-lake-nakuru-national-park-58005/**

2012: 100 animals were killed in outbreaks in Turkana.
http://www.standardmedia.co.ke/article/2000066907/anthrax-outbreak-kills-100-animals-in-turkana

2011: 11 giraffes were found dead in a 3 month period at the Mwea National Reserve.
https://www.dovepress.com/an-outbreak-of-anthrax-in-endangered-rothschildrsquos-giraffes-in-mwea-peer-reviewed-article-VMRR

2006: 2 people died following an anthrax outbreak in Maragua and Samburu districs.
http://www.controlofbiohazards.com/Documents/AnthraxJan2006--Dec2006.pdf

Madagascar

No recent information available on PubMed or the internet (English and/or French).

Malawi

No recent information available on PubMed or the internet.

Mali

No recent information available on PubMed or the internet (English and/or French).

Mozambique

No recent information available on PubMed or the internet (English and/or Portuguese).

Rwanda

No recent information available on PubMed or the internet (English and/or French).

Senegal

No relevant data on PubMed

There are references on internet about the government supporting vaccination campaigns for anthrax in different years, but no data on outbreaks or prevalence.

South Africa

Anthrax is endemic in parts of southern Africa and is commonly reported in livestock and wildlife.

2014: Northern Cape Province - During March 2014 there were reports of an unusual increase in deaths among sheep and goats in Sanddrift (Namakwa District), close to the Namibian border and anthrax was confirmed. The last recorded animal anthrax outbreak in this area was in 2006, and occurred in Kuboes, a small town neighbouring Sanddrift. One human case of probable cutaneous anthrax was identified, and 19 persons exposed to anthrax-contaminated goat and sheep carcasses were given antibiotic prophylaxis and monitored.
http://www.nicd.ac.za/assets/files/NICD-NHLS%20AnthraX.pdf

2014: Northern Kruger National Park, Limpopo Province - An outbreak of anthrax among wildlife was reported in the northern Pafuri area of the Kruger National Park. Although this area is known to be endemic for anthrax with wildlife cases reported every year, there was a sudden dramatic increase in the number of anthrax cases during February 2014. Affected wildlife species included impala, kudu, nyala and Burchell's zebra.
http://www.nicd.ac.za/assets/files/NICD-NHLS%20AnthraX.pdf

Tanzania

Anthrax is widespread in Tanzania, affecting livestock and wildlife.

2014: Anthrax was detected in hippopotamuses, soil and humans.
http://www.ojvr.org/index.php/ojvr/article/viewFile/722/1000

2013: Reports of anthrax from several districts of Arusha and Kilimanjarao regions, linked to livestock.
http://promedmail.chip.org/pipermail/promed-eafr/2013-July/001349.html

Outbreak in Moshi – Kilimanjaro. 10 cases associated to 1 dead cow.
http://aslm.org/aslm2012/images/docs/Friday-December-7th-2012/Oral-Posters/Role-of-Laboratory-in-Outbreak-Investigations/2.%20Mura%20Ngoi.pdf

There is an interesting thesis about the suitability of the anthrax vaccine for cattle, sheep and goats distributed by CVL, Dar es Salaam, but unfortunately, a full copy of the thesis is not available.
http://dspace.library.uu.nl/handle/1874/231225

Uganda

Sporadic anthrax outbreaks have occurred in and around Uganda's Queen Elizabeth National Park (QENP) for years, affecting wildlife, domestic animals, and humans.

Recently there have been several outbreaks among wildlife in Uganda's Queen Elizabeth National Park (QENP). The 2004-2005 QENP outbreak killed over 450 animals: 306 hippopotami representing 11.63%, 63 zebras representing 1.47%, 60 buffaloes representing 0.9%, thirteen warthogs representing 0.69%, twelve kobs representing 0.07%, three waterbucks representing 0.09% and five elephants representing 0.02% died (Wafula, 2008 **http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2028.2007.00796.x/abstract**). Such a cycle of disease recurrence results in higher infection risk among towns and villages near the park.

Most recently, there was an outbreak in 2011 in Sheema District (more than 50 km from the park) and involved no wild animals. This outbreak temporarily shut down meat, dairy, and livestock markets and claimed the lives of two humans and nine livestock [24].

Zambia

It has been demonstrated highly pathogenic *B. cereus* is present in Zambia. Cases of anthrax-suspected deaths in wildlife have been reported every year in Lower Zambezi National Park; *B. cereus* was implicated as the cause in at least some of these cases. Zambia may be a high-risk country for highly pathogenic *B. cereus* infection outbreaks [25].

August 2011: an anthrax outbreak occurred among *Hippopotamus amphibius* hippopotamuses in the Chama district of the eastern province of Zambia. Over 80 hippopotamuses died after showing signs of infection with *B. anthracis*. Following the deaths of the hippopotamuses, 521 suspected human cases resulting in 6 deaths were reported [26].

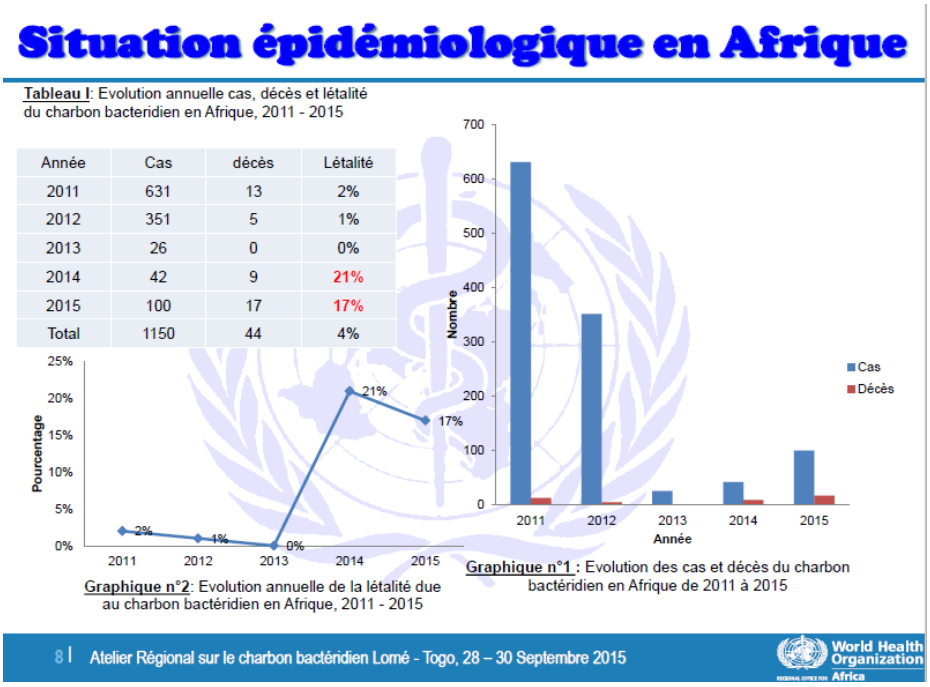
Nov 2010: An anthrax outbreak occurred in five villages of Sesheke district in Western Zambia. The average herd size of infected cattle in affected villages was estimated at 121.8 (95% CI 48.8-194.8). Individual mortality per herd varied between 1.70% (3/179) and 20.25% (6/79). In humans, the disease only affected three people and was characterized by cutaneous carbuncles. The ratio of infected persons per number of infected carcasses varied between 1:37 and 1:49 in affected villages while the overall ratio of people at risk to the number of carcasses was 42:1 indicating that despite availability of a large number of carcasses, human contact with infected carcasses was low [27].

1999 – 2007: Anthrax is endemic throughout the upper Zambezi floodplain (Western Province). Retrospective data for the period 1999 to 2007 showed that a total of 1,216 bovine cases of anthrax were reported. During the same period, 1,790 human anthrax cases and a corresponding case fatality rate of 4.63% (83/1,790) was documented in the upper Zambezi floodplain. Occurrence of human cases was highly correlated with cattle outbreaks ($r = 0.94$, $p < 0.001$) [28].

1989-1995: Anthrax is endemic in Western and North-western Provinces of Zambia. The disease occurs throughout the year and impacts negatively on the economy of the livestock industry and public health in Zambia. During 1989-1995, there were 1626 suspected cases of anthrax in cattle in Western province and of these 51 were confirmed. There were 220 cases of human anthrax cases in 1990 alone and 248 cases during 1991-1998 with 19.1% and 7.7% case fatality rates, respectively [29].

3- Human data in Africa:

Data reported by Dr Sodjinou (WHO) on the human anthrax situation in Africa during the Regional meeting on Anthrax in West Africa, organized by FAO (with the collaboration of AU-IBAR, CDC, OIE and EUMOA) in Togo in Sept 2015 (presentations are not available on line, but were obtained by direct request to FAO).



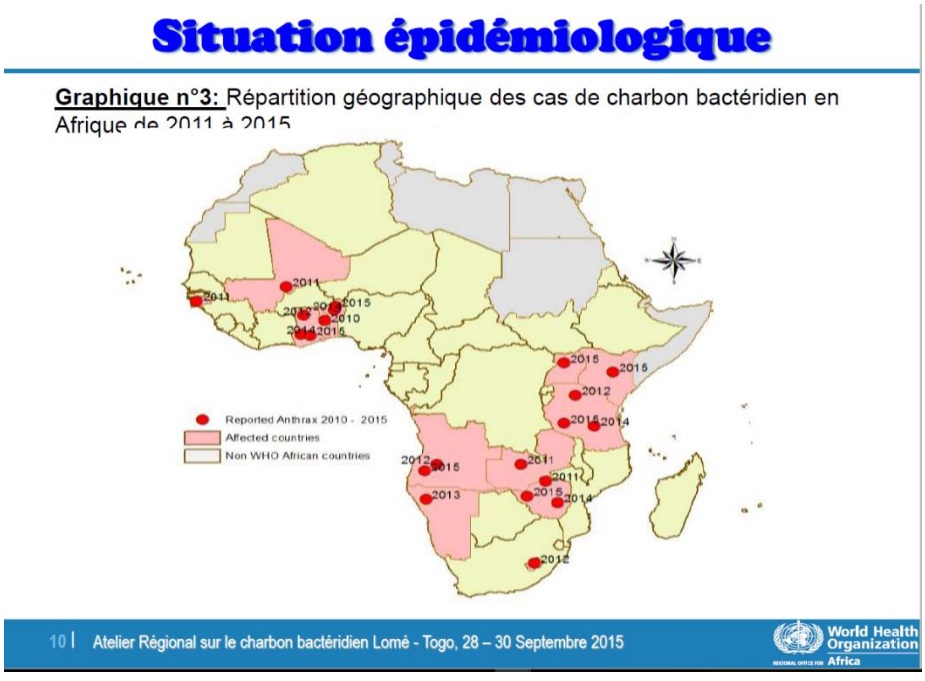


Figure 5: Epidemiological situation of human anthrax in Africa. (Source: Dr Sodjnou, Togo 2015)

Prevalence data by country (from 2000 onwards)

Due to the nature of the disease (peracute and acute manifestations, with very high mortality as actually the disease needs to kill the animals so it can propagate, and its manifestation as sporadic cases in endemic regions), it is very difficult if not impossible to measure prevalence. An indirect way to measure prevalence in animals would be to measure prevalence in humans, but again, the disease is very much underreported, so it is very difficult to estimate.

The research done showed very little information for each of the countries of interest. Sources used were PubMed and internet engine searches in English (and French for fievre charbonneuse when applicable).

Real prevalence data was only found for Bangladesh, Tanzania and Zambia. For all the other countries, the information available refers to number of outbreaks or number of cases, which relates to incidence and not to prevalence.

Bangladesh

Prevalence information for Bangladesh is shown in Table 6.

Table 6: Estimated number of diagnosed cases, prevalence, death cases and vaccination coverage of anthrax in livestock in Bangladesh, 2010–2012. Source: Mondal et al, 2014 [14]

Disease	2010	2011	2012	Total
Anthrax				
Diagnosed cases	2,174	1,668	2,095	5,937
Prevalence rate, % (95% CI*)	0.14 (0.13–0.15)[δ]	0.09 (0.08–0.09)	0.17 (0.16–0.18)	0.13 (0.12–0.13)
Death cases	433	173	195	801
Case fatality rate, % (95% CI)	19.92 (18.23–21.61)	10.37 (8.90–11.84)	9.31 (8.06–10.56)	13.49 (12.62–14.37)
Vaccination	2,602,967	3,417,136	3,325,525	9,345,628
Vaccination rate, % (95% CI)	6.11 (6.10–6.12)[†]	8.02 (8.01–8.03)	7.81 (7.80–7.82)	7.31 (7.31–7.32)

Tanzania

Source: Hampson et al, 2011 [30]. **http://www.ncbi.nlm.nih.gov/pubmed/22318563**

In a study done in the Serengeti ecosystem, seroprevalence was consistently high in carnivores (90% and 57% overall seropositivity in Serengeti and Ngorongoro Crater lions respectively, and 87% seropositivity in Serengeti spotted hyenas) and significantly lower among herbivores (46% and 14% seropositivity in Serengeti and Ngorongoro Crater buffalo, 19% and 4% in Serengeti and Ngorongoro Crater wildebeest), with no seropositive zebras. Overall seroprevalence was lower in Ngorongoro Crater wildlife populations than in Serengeti.

Human anthrax case reports were sporadic and originated exclusively from pastoralist villages in the NCA and Loliondo division, consistent with areas of high seroprevalence in domestic dogs and cases in wildlife and livestock.

Zambia

Source: Munang'andu et al, 2012 [27] **http://www.ncbi.nlm.nih.gov/pubmed/22885011**

In an anthrax outbreak in five villages of Sesheke district in Western Zambia, the prevalence of the disease in cattle was estimated at 7.4% (45/609) while the average herd size of infected cattle in affected villages was estimated at 121.8 (95% CI 48.8-194.8). In humans, the disease only affected three people and was characterized by cutaneous carbuncles.

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

The economic and social impacts of anthrax are due to the human, livestock and wildlife disease. The disease itself and the standard control methods have serious economic, biosecurity, and conservation implications. It is important to note that anthrax can considerably affect wildlife.

Humans

Anthrax is an important zoonoses, and the WHO estimates that between 20,000 and 100,000 human cases occur globally per year.

B. anthracis has always been high on the list of potential agents with respect to biological warfare and bioterrorism. It has been used in that context on at least 2 occasions, and been the named agent in many threats and hoaxes. The information available regarding the impact of anthrax in humans, is focused on the impact of anthrax as a bioterrorist threat. In 1997, Kaufmann et al [31], constructed a model that compared the impact of three classic agents of biologic warfare (*Bacillus anthracis*, *Brucella melitensis*, and *Francisella tularensis*) when released as aerosols in the suburb of a major city. The model showed that the economic impact of a bioterrorist attack can range from an estimated $477.7 million per 100,000 persons exposed (brucellosis scenario) to $26.2 billion per 100,000 persons exposed (anthrax scenario). Rapid implementation of a post-attack prophylaxis program was identified as the single most important means of reducing these losses.

The economic impacts of a wide area release of Anthrax was also evaluated by the US Department of Homeland Security in 2009 (**http://www.pnl.gov/main/publications/external/technical_reports/PNNL-18448.pdf**). The different cost categories included are seen in Figure 6, and were:

- **Loss of Life** includes costs associated with compensation for lost earnings, which may be paid by insurance policies, the government, businesses, or charities; or the costs may be absorbed by individual families. There may also be costs associated with lost productivity from loss of life as organizations are forced to operate without essential staff. A potential cost could come in the form of liability for loss of life if building owners were thought to have provided inadequate protection.

- **Healthcare** refers to costs associated with diagnosis and treatment of the sick or injured and responding to the worried well.
- **Damage and Loss of Property** refers to costs associated with damaged property and lost assets that require replacement. Other costs may include insurance losses on property. For the federal government, there may also be costs associated with allocation of grant funding to state and local agencies and other critical responders.
- **Evacuation and Return** costs are those associated with physically moving citizens and businesses away from the contaminated and possibly surrounding area, either voluntarily or through planned evacuation. There will be costs for government to keep people out of unsafe areas as well. And when it is time to repopulate the evacuated areas, there may be costs to support the return to homes and businesses. Finally, there may be costs associated with temporarily relocating people and businesses and incentivising them to return to their buildings.
- **Decontamination or Decommissioning** refers to the broad set of costs associated with cleaning up buildings, homes, and critical infrastructure that might have been contaminated in the attack. It may also be determined that an area/building is not a good candidate for decontamination due to economic, legal, or other factors, and may be decommissioned instead. Examples of decontamination costs include sampling and testing, remediation labour, supplies and equipment costs, and waste disposal. There could also be costs resulting from liability for exposure during clean-up or for inadequate clean-up.
- **Other Indirect Economic Losses** captures other means through which value could be lost to government and the private sector as a result of the disruption to the economy. The loss of income will have a major impact on all stakeholder groups, and could ultimately cause defaulting on loans or tightened access to credit. The government's main source of income is tax revenue, which will be affected when its residential and commercial population evacuates or is impaired. Businesses earn revenue from commerce; beyond the direct loss of commerce from businesses forced to close or relocate, factors such as loss of consumer confidence will have a negative effect on businesses throughout the regional economy. Finally, rising civil unrest as a result of increasing social stratification, a growth in potential hate crimes and discrimination may result in other costs to property owners and society as a whole.

Livestock

The mortality rate for anthrax varies with the species. Clinical infections in ruminants and horses are usually fatal; pigs often recover.

With the development of the Sterne spore vaccine, a sharp decline in anthrax outbreaks in livestock occurred during the 1930-1980 era. However, a resurgence of this disease in livestock has been reported in some regions, where complacency, a false sense of security or other causes have hindered vaccination.

The main losses in case of an outbreak would be from mortalities, which can be high, and losses due to an inability to trade while quarantine restrictions are in place. There would be an increased cost from vaccinating the animals. There is also a considerable impact on the producer due to resources and significant effort required for disposal of affected carcases (burnt in-situ) and site decontamination.

At national level, for countries with sporadic cases, it is unlikely that outbreaks would continue for more than 2 months from the start to the lifting of the restrictions, and the likelihood of there being long-term economic effects would be low.

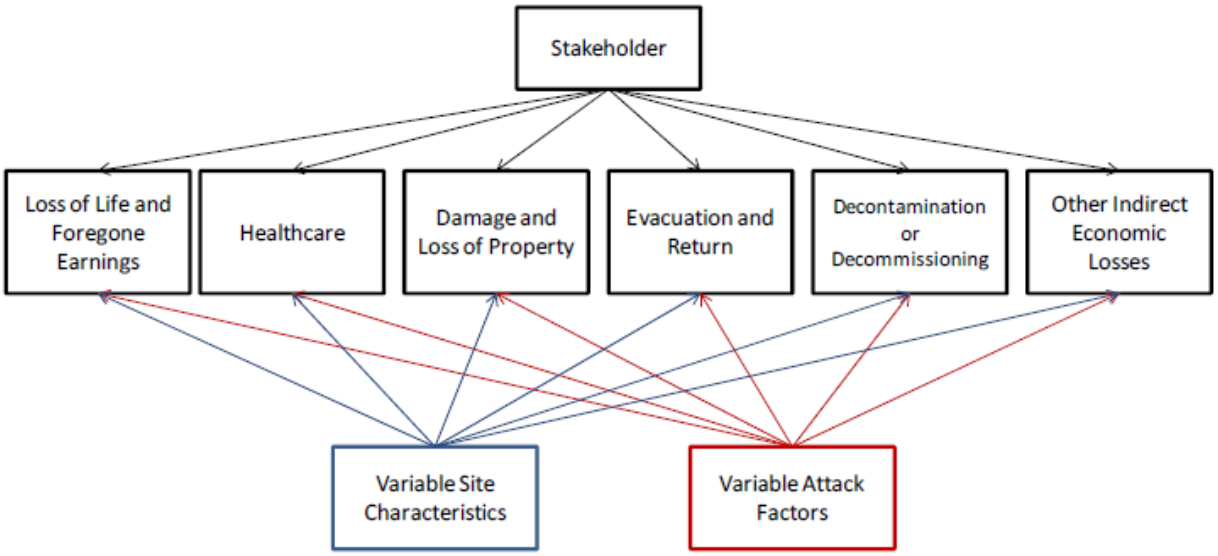


Figure 6: General Framework for analysis of economic impact of a wide area release of Anthrax (Source: Judd et al, 2009 – Economic impacts of a wide area release of anthrax).

Analysis by the World Bank

The World Livestock Disease Atlas – a quantitative analysis of global animal health data [32], published by the World Bank (with cooperation of OIE and FAO) in 2011 is an attempt to understand which livestock diseases cause the heaviest losses, which countries suffers the worst disease-related losses and which livestock species are most affected.

http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2012/02/17/000356161_20120217030841/Rendered/PDF/668590WP00PUBL00Livestock0Atlas0web.pdf

The World Livestock Disease Atlas bases its analysis on the Livestock Units (LSU). Each species has a LSU value, and the losses of LSU have been given a value. See Figure 7. For more information on the methodology description, please refer to the World Bank Atlas itself (pages 6 & 7). Anthrax is one of the top 10 diseases causing losses for cattle, buffalos and small ruminants, as shown in Figure 8. However, looking at the data in detail, there are few data from sub-Saharan Africa and Asia.

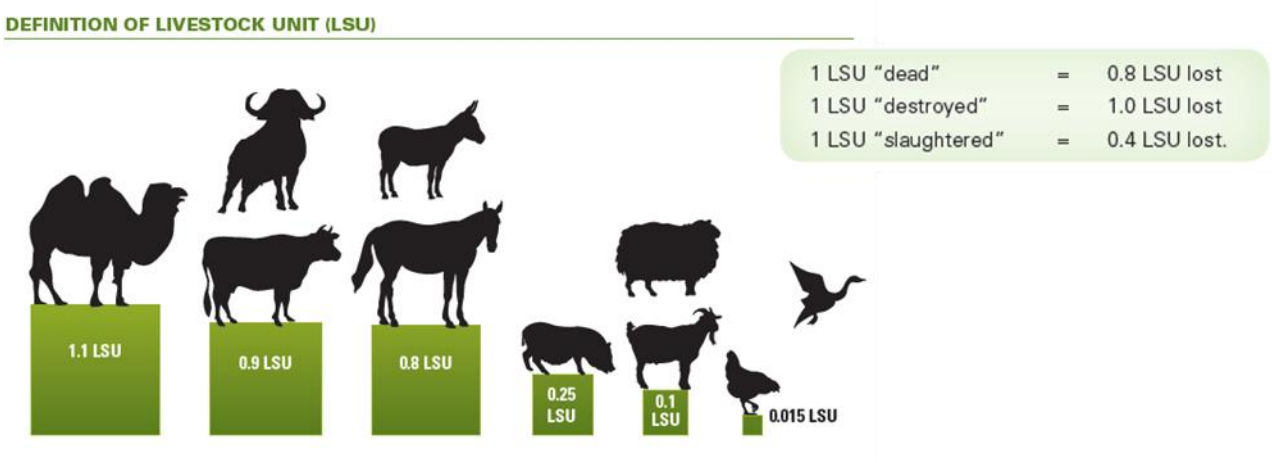


Figure 7: Livestock Units. Source: World Livestock Disease Atlas – The World Bank, 2011 [32].

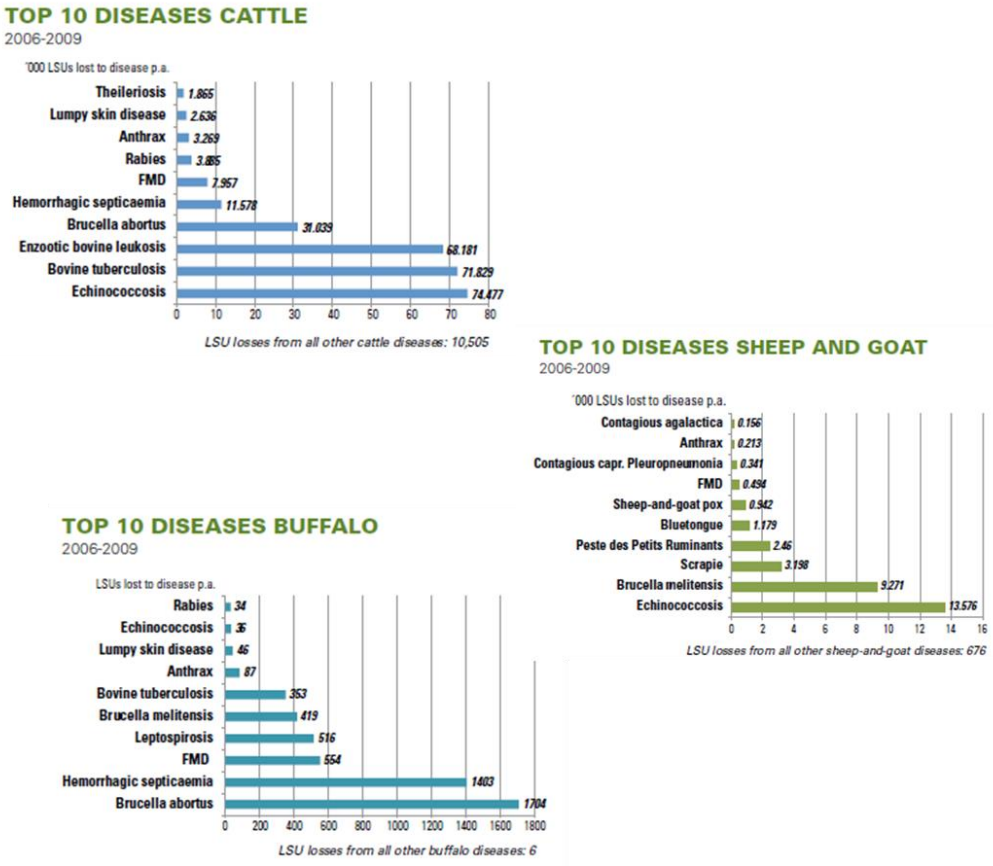


Figure 8: Top 10 diseases in terms of LSU losses for cattle, buffalo, and sheep & goats. Source: World Livestock Disease Atlas – The World Bank, 2011 [32].

Wildlife

Major epidemics of anthrax periodically flare up in African wildlife conservation areas such as the Queen Elizabeth National Park in Uganda, the Omo-Mago National Park in Ethiopia, the Selous Nature Reserve in Tanania, the Luangwa Valley in Zambia, the Etosha National Park in Namibia, the Kgalagadi Transfrontier Park in South Africa and Botswana, and the Vaalbos and Kruger National Parks in South Africa [33]. In the Kruger National Park, a symbiotic relationship between anthrax and the complex of ecosystems exist. Outbreaks in National parks, can present a persistent risk to surrounding livestock, as well as a public health.

In multi-species African conservation areas, carcass counts and analyses demonstrate that certain herbivores are over or under represented in relation to their population numbers and densities, indicating varying degrees of susceptibility and or behavioral vulnerability to infection. In general, the spiral horned antelope, and buffalo are over-represented, while the Acelpahines, zebra and impala are under-represented.

However, the impact of the disease can be considerable. As an example, in 2004, an outbreak in Malilangwe Wildlife Reserve in Zimbabwe killed almost all of the approximately 500 kudu in the reserve, as well as large numbers of other wild ruminants. Other species badly affected were nyala, bushbuck, waterbuck and roan antelope, which suffered losses of approximately 68%, 48% 44% and 42% of their populations respectively. Buffalo were also badly affected, and although their populations suffered only 6% losses, the number of deaths ranked second highest after kudu [34].

In many natural parks in Africa, anthrax is considered indigenous and an integral part of the ecosystem. The policy is to institute active control measures only if it affects biodiversity or a human intervention is providing impetus to the disease. Some animals like cheetah and black rhinoceros are endangered species, so they qualify for direct control measures such as vaccination. At Etosha National Park (Namibia), black rhinoceros have been vaccinated since the 1970s [35].

Impact on specific focus countries

There are no published reports about the detailed specific economic and social impact of anthrax in the countries of interest. However, some publications have mentioned its impact:

Bangladesh

A human outbreak occurred from April to September 2011, affected mostly the two North-Western districts of Sirajganj (61 cases) and Pabna (32 cases). Additionally, the districts of Bogra, Meherpur and Tangail had 28, 39 and 14 cases of anthrax respectively. Due to vaccine coverage in the preceding years, the number of livestock deaths was minimal and only a few infected slaughtered animals wer
e held responsible for human transmission during the outbreak. Fortunately there were no anthrax-related human deaths but meat sales drastically declined due to a lack of consumer confidence, and anthrax created

mass havoc with significant economic losses related to cattle farming. Although there was no known case fatality, people panicked and mass immunization of livestock was demanded by concerned sections [15].

Uganda

An outbreak in 2011 in Sheema District temporarily shut down meat, dairy, and livestock markets and claimed the lives of two humans and nine livestock [24].

Disease Prevention and Control Methods

Anthrax is an internationally reportable disease. Being a notifiable disease by law in most countries, anthrax control procedures are usually prescribed and enforced by government veterinary services.

Anthrax control measures are aimed at breaking the cycle of infection and consist basically of a surveillance system, prophylactic procedures (immunization, treatment and disinfection), and disease regulatory actions (quarantine, proper disposal of carcasses, decontamination of the site and disinfection of the items used to test and dispose of the carcasses, treatment and immunization). If a potential infectious source is known to exist, it should be eliminated without delay. The best method to dispose of the carcasses is incineration.

Treatment (Control)

Bacillus anthracis is susceptible to most antibiotics; however, because of the peracute nature of anthrax disease in herbivores, by the time the animal shows clinical signs it is usually too late to treat with antibiotics. During a herd outbreak in cattle, it may be useful to treat any sick or febrile animals as well as any suspect incubating cases with long-acting penicillin or tetracyclines. The WHO/OIE/FAO recommends the use of penicillin with streptomycin. Anthrax has a less acute course in carnivores, and during outbreaks in southern Africa, lions and leopards with typical clinical signs (swollen faces and lips) have been successfully treated with large doses of long-acting penicillin [1]. However, a few countries, do not permit antibiotic treatment, requiring slaughter with appropriate disposal instead.

Recommendations from AU-IBAR: (http://www.au-ibar.org/anthrax)

The first sign of anthrax in a herd is unexpected death of one or more animals. Following this first incident of anthrax in a herd, the remaining animals should be moved immediately from the site where the index case(s) died. Animals should be checked at least three times daily for 2-weeks for signs of illness (rapid breathing, elevated body temperature) or of submandibular or other oedema. Any animal showing these signs should be separated from the herd and treated with antibiotics immediately. Intravenous sodium benzylpenicillin according to manufacturer's instructions, usually in the range 12,000-22,000 units per kg of body weight. This should be followed 6-8 hours later by intramuscular injection of long acting benethamine penicillin for which

manufacturers' instructions usually recommend dose within range 6000-12,000 units per kg of body weight. Alternatively, another appropriate long-acting preparation such as Clamoxyl® (15 mg/kg), a long-acting preparation of amoxycillin can be administered and are normally successful. If long-acting preparations are unavailable, procaine penicillin (dose recommended by manufacturers is usually 6000-12,000 units/kg) can be used for intramuscular injection but should be administered again after 24 and 48 hours. Streptomycin acts synergistically with penicillin and penicillin/streptomycin mixtures are available commercially but involve an extra cost. Recommended doses of streptomycin to be administered together with penicillin intramuscularly are 5-10 mg per kg body weight in large animals and 25-100 mg per kg body weight in small animals.

If there is concern that the antibiotic treatment will not control the outbreak, the herd should be vaccinated.

Live spores constitute the active ingredient of the vaccine and therefore treatment should not be done simultaneously with vaccination. Deaths usually cease within 8 to 14 days of vaccination. Herd quarantine can be lifted 21 days after the last death. Decontamination of the site(s) where the index case or other case(s) died should be carried out.

In certain countries, treatment is not permitted and slaughter is mandatory.

Prophylaxis (Prevention)

Prophylactic treatment may also be indicated prior to moving animals out of an outbreak zone. These animals can then be vaccinated at their destination a minimum of 14 days after the date of the antibiotic treatment.

Options and strategies for control programs at national, sub-national or regional level

Control measures for anthrax are aimed at breaking the cycle of infection and involve four main components:

1. Discontinuation of infection source and site quarantine:
   In outbreaks having a defined source, discontinuing this source is an essential first step. If the infection can be traced to feeding for example, the feed source should be immediately withdrawn from the index farm and from all others that received it. Moving other animals from the affected area is an important early action. If flies are suspected of being important vectors, fly control should be considered. A contingency plan for the prevention and control for anthrax is included as Appendix VI in the WHO anthrax guidelines (WHO, 2008). It recommends that affected premises should be quarantined for at least 20 days (OIE incubation period) after the last case. Any susceptible livestock, or risky items (carcasses, hides, skins, etc.) moved from the premise should be traced and the appropriate action taken.

2. Correct disposal of carcasses of animals that have died of anthrax:
   The carcasses should not be opened, and they should be incinerated, rendered or buried. The preferred

method of carcass disposal is in situ incineration by a method that ensures thorough scorching of the contaminated ground in the vicinity of the carcass. Practical details are given in Appendix III of the WHO anthrax guidelines (WHO, 2008). Where lack of fuel prevents this, burial 1.5 to 2 m below ground may be the only option. Burial is not ideal, as it is labour intense and requires a mix of chloride and lime in the soil. Numerous reports exist, however, of fresh outbreaks occurring after disturbance of known or suspected anthrax burial sites, and burial should always be discouraged in favour of incineration where possible. Some countries spray 5% formaldehyde on and around the carcass to deter scavengers. Where local regulations permit it, transfer of a carcass to a body bag and transport to a permanent incinerator may be considered. Care must be taken to avoid spreading contamination while manipulating the carcass.

3. Decontamination by appropriate methods of the sites where the animals died and of any materials subsequently contaminated:
   Appropriate decontamination methods include dry heat, autoclaving, incineration, fumigation with sporicidal fumigants (formaldehyde, ethylene oxide), disinfection with sporicidal disinfectants (hypochlorite, formalin, glutaraldehyde, hydrogen peroxide, peracetic acid) or irradiation. It has been suggested that dry heat at 160°C for one hour or irradiation at ±20 kGy is sufficient depending on the degree of contamination and the bulk of the material. Different procedures and, where relevant, chemicals will be appropriate for different materials and circumstances. Hypochlorites are not suitable for metal objects or materials with a high organic content. Formalin is often the best and easiest sporicidal disinfectant to apply but is hazardous to operators and animals. Soil can be incinerated, but whether this is practical depends on the quantity of contaminated soil present. Where contaminated sites are too large to practically decontaminate, the only approach may be to isolate them, for example by concreting them over.

4. Vaccination where there is risk of the outbreak spreading:
   Livestock vaccines in almost all countries are suspensions of spores of an attenuated strain of B. anthracis usually with saponin as an adjuvant. See Section 6 for more details on vaccines used.

In countries with well-established veterinary services and public health monitoring, anthrax outbreaks are rapidly contained by the imposition of standard control measures. EU standards are the establishment of a 3 km radius of protection (vaccination) after an outbreak, and 10 km radius surveillance.

In countries in which the disease is not well controlled, regular outbreaks of anthrax can become serious epidemics of both animals and humans. For example, when civil war interrupted normal vaccination and regulatory controls in Zimbabwe in the late 1970s, a massive outbreak in 1978 resulted in more than 10,000 human cases and untold number of deaths in cattle.

In some countries like South Africa, there are laws for compulsory annual vaccination (Animal Diseases Act No.35 of 1984 which aims at the establishment of an immune population through the compulsory annual vaccination of all cattle). In the past, government veterinary authorities shouldered the responsibility of

vaccination and achieved considerable success. However, when responsibility shifted to the farmer, there was a sharp decline in number of animals vaccinated annually.

Control of anthrax in wildlife

In the major game reserves, such as the Kruger National Park, most of the control measures, as used for livestock, are difficult, if not impossible, to apply and/or enforce. In addition, anthrax, being considered indigenous in a natural and integral part of the ecosystems of some of these areas, makes it debatable whether active control measures should actually be instituted. In the national parks of South Africa, the policy is to institute active control measures against anthrax only if it affects biodiversity negatively (e.g. by threatening the survival of low density or threatened species), and/or where the actions of humans (such as fencing or the provision of artificial watering points), are providing unnatural impetus to an outbreak. As a general rule of thumb, the bigger and more natural and self-sufficient an area is, the less control measures should be implemented.

Vaccinating wild animals can be very difficult, being by massive capture and use of a chute, or by using ground vehicles, hides or helicopter platforms. This last form requires remote injection by means of “drop-out” darts or biodegradable ballistic implant projectiles. The need to repeat vaccination annually is daunting. There is a definite need for the development of an effective oral vaccine for ranched and habituated wildlife, which can be delivered by water or feed.

Apart from vaccination, anthrax control procedures in wildlife include the fencing-off or burning of known anthrax contaminated vegetation, the location and covering and/or incineration of carcasses as soon as possible so as to prevent their dismemberment by scavengers, and the replacement of natural waterholes by concrete drinking troughs in which the water can be disinfected or it can be drained.

Disease situation and government policies by country

Tables 7 and 8 below have been completed with the information received from the questionnaires sent to the DG and DVS. For a list of respondents, please see Annex 3.

Table 7 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 8 refers to vaccination.

The definitions that were given to the respondents are:

[1]Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a program which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 7: Official status, official programs and treatment for anthrax in the countries of interest. Information provided by the questionnaires sent to the DG/DVS as part of this monograph.

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) (if yes, active or passive)	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
ASIA					
Bangladesh	Yes	Yes, passive	No	Yes	Yes
India					
Indonesia					
Myanmar (Burma)	Yes	Yes, passive	Yes	No	Yes
Nepal	Yes	Yes, passive	No	No	No
Vietnam	Yes	Yes, passive	No	No	Yes
AFRICA					
Burkina Faso					
Côte d'Ivoire (Ivory Coast)	Yes	Passive, but active when there is an outbreak	Yes	No	
Ethiopia					
Kenya	Yes	Yes, active and passive	No	Yes	No
Madagascar					

Malawi	Yes	No	No	No	No
Mali	Yes	Yes, passive	Yes	No	No
Mozambique					
Rwanda	Yes	Yes, active and passive	Yes	Yes	No
Senegal					
South Africa					
Tanzania	Yes	Yes, passive	No	Yes	No
Uganda	Yes	No	No	Yes	No
Zambia	Yes	Yes, passive	Yes	Yes	No

Table 8: Vaccination for Anthrax in the countries of interest.
Information provided by the questionnaires sent to the DG/DVS as part of this monograph.

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
ASIA				
Bangladesh	No	Combination (Government subsidy, owner pays service charge)	Official	Cattle, buffalo. To some extent, goat and sheep
India				

Indonesia				
Myanmar (Burma)	Yes	Government	Official	Cattle
Nepal	No	N/A	N/A	N/A
Vietnam	No	Farmers	Both	Cattle
AFRICA				
Burkina Faso				
Côte d'Ivoire (Ivory Coast)	No	Government in case of outbreaks. If not, farmers.	Official	Cattle, sheep and goats
Ethiopia				
Kenya	No	Farmers	Both	Cattle, sheep, goats
Madagascar				
Malawi	No	N/A	N/A	N/A
Mali	No	Combination	Official	Cattle, sheep & goats
Mozambique				
Rwanda	Yes	Farmers	Official	Cattle
Senegal				
South Africa				
Tanzania	No	Farmers	Both	Cattle, sheep & goats
Uganda	No	Government & farmers	Both (government & private)	Cattle
Zambia	No	Farmers	Both	Cattle

Regional efforts

It has been noticed than in 2014, UEMOA (the West African Economic and Monetary Union) supported an anthrax control effort in West Africa. It included Benin, Burkina Faso, Ivory Coast, Guinea Bissau, Mali, Niger and Togo (Senegal to start in 2016). In Ivory Coast, 36 vaccinators were mobilized, and they vaccinated a total of 363,711 animals including cattle, sheep and goats between Nov 2013 and January 2014. Source: Dr Domagni presentation during the Regional meeting on Anthrax in West Africa, organized by FAO (with the collaboration of AU-IBAR, CDC, OIE and EUMOA) in Togo in Sept 2015 (presentations are not available on line, but were obtained from Dr Bedane from FAO).

Vaccines Available

Current vaccine types

Pasteur demonstrated protection against anthrax by immunisation in 1881 using a heat attenuated strain. However, problems with declining potency and variations in virulence led to the search for a more effective and stable vaccine. There were 2 types of Pasteur vaccine, type 1 and type 2. The Pasteur type 1 strain was used until relatively recently in Italy to vaccinate goats and horses.

Most anthrax vaccines in use in the world at present, utilize the toxigenic, non-capsulating *B. anthracis* 34F2 isolated in 1937 by Max Sterne at Ondesterpoort in South Africa, from a subculture from a case of bovine anthrax. This variant has lost the pX02 plasmid which codes for capsule formation (pX01+, pX02-). It is used essentially as originally formulated with approximately 10^7 spores/ml suspended in 1.5% saponin in 50% glycerol-saline. Some laboratories use aluminium hydroxide gel instead. In the past, this strain has also been wrongly called “Weybridge” strain. The basis of protection is the development of antibodies to the Protective Antigen.

There are also other livestock vaccines used in specific countries or regions. The anthrax strain 1190R was developed in Romania, more or less at the same time than the Sterne strain, and it is still being used locally. The anthrax strain 55 is used mainly in Russia, Central and Eastern Europe. Both strains are very similar to the Sterne stain (pX01+, pX02-). Italy has also been using the Carbosap vaccine, produced from a pX01+, pX02+ strain.

The OIE manual mentions only the Sterne 34F2 and the strain 55 vaccines. The OIE recommends a minimum 2-10 x10^6 culturable spores per dose for cattle, buffaloes and horses, and no less than 1-5x10^6 culturable spores per dose for sheep, goats and pigs. However, protection tends to be less than 100% in animals if they have only received one dose, and the dose was less than 10^7 spores. Recommended doses may vary by country and manufacturer. For example, in Australia the only registered anthrax vaccine delivers the following minimum doses: Cattle: 4 million viable spores, Sheep and goats: 2 million viable spores; in the United States the dose is 2 million viable spores per doses [36].

Sterne 34F2 vaccine

The live spore Sterne vaccines is, for all practical purposes, non-pathogenic in most domestic and wild animal species. It causes few adverse reactions in cattle and sheep (they might have an elevated temperature 12-36

hours after vaccination, causing reduced milk yield). However, it appears to retain a degree of virulence for certain species, and severe reactions can occur in goats and llamas. In such species, 2 inoculations one month apart, with the first one being one quarter of the standard dose and the second a full standard dose, are recommended (not all manufacturers recommend this). Llamas are often mentioned together with goats, but the evidence is limited to one single publication in 1987. It is possible that the reaction in goats is due to the quality of the saponin component, as it is known that goats react to certain saponins (Dungu, personal communication). Deaths associated with anthrax vaccine, have been reported recently in miniature horses in Canada [37].

In horses, being slow to develop immunity (one month or more) 2 standard doses, one month apart and a single annual booster is recommended. Two initial doses approx. 8 weeks apart have been found necessary to develop dependably measurably antibody titres in zebra. It the other species, a single vaccination is usually effective for 6-12 months, provided that animals receive the full dose and because the active ingredient is live spores, the animals are not under antibiotic treatment within 10-14 days before or after vaccination. In heavily contaminated areas, two vaccinations, 2-3 weeks apart are recommended. Annual revaccination is generally considered to be adequate to ensure permanent protection in most situations. Effective immunity generally develops within a week of vaccination, although as already mentioned, in horses it may take a month or more.

There are many manufacturers of the Sterne 34F2 vaccine worldwide. The most common adjuvant is Saponin, but aluminium hydroxide is also used. Some manufacturers also include glycerine.

Vaccine production issues

Uncapsulated *B. anthracis* variants may lose their immunogenicity on subculture. It is therefore important that standardized procedures for the manufacturing of anthrax vaccines be used. There are also considerable differences in vaccine quality and it might be partially due to the quality of the glycerine and saponin used. Issues relating to vaccine quality have been reported (as an example, comments on the quality of the vaccine manufactured in Tanzania have been included in a PhD thesis from Pilot M.A in 2012 called “The suitability of the anthrax vaccine for cattle, sheep and goats distributed by CVL, Dar es Salaam, Tanzania”, but unfortunately the full text is not available even by request. The abstract can be seen at: **http://dspace.library.uu.nl/handle/1874/231225**).

The Sterne strain loses the ability to produce integral toxins. They have subsequently recovered intact toxin-producing bacteria by infecting mice and re-isolated the bacterium from brain. In this way they were able to revitalize the Sterne strain.

Vaccine failures may occur as a result of poor quality or low potency vaccine, animals not receiving the correct dose, animals receiving such a high infective dose that the body’s immune system in overpowered, animals being under antibiotic effects, or an as yet unexplained poor immunogenic reaction of individuals. As for potential vaccine failure related to the local lineages, please see *B. anthracis* lineages in Page 7.

Pasteur strains

The attenuation of the Pasteur vaccines is mainly due to the loss of the plasmid pXO1. These vaccines were obtained from Pasteur cultivating pathogenic strains of *Bacillus anthracis* at a temperature of 42°C. This treatment had caused the disappearance in the pathogenic strain of the plasmid pXO1 and consequently of all the toxic factors encoded in it. The absence of pXO1 justifies the attenuation of the strain, since there is no production of EF, LF and PA, but also raises serious questions about the real protective capabilities of these vaccines for the failure to produce even the PA.

There are two types of Pasteur vaccines. Type 1 is pathogenic for mice and apathogenic for the guinea pig; they are obtained by growing the bacterium at temperatures of 42° - 43°C for 15-20 days; Type 2 is pathogenic for mice and guinea pig and apathogenic for the rabbit; they are obtained by growing the bacterium at temperatures of 42° - 43°C for only 10-12 days.

The Pasteur Type 1 vaccine was used in Italy until 2006, and it was produced by the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (IZS PB). The Pasteur Type 2 is only used in China.

Italy: Carbosap

The strain was obtained in the laboratories of the "Istituto Vaccinogeno di Asmara" during the Second World War, by the research group led by Prof. Cilli. Introduced in Italy by Prof. Boat it was used between 1949 and 2006 in the campaigns of vaccination against anthrax and has been of great contribution in the fight for the eradication of anthrax in Italy. The vaccine strain Carbosap possessed both plasmids, pXO1 and pXO2, and had the capsule and was able to produce all three toxins. The presence of all plasmids revealed its similarity with three other atypical Pasteur vaccine strains (# 1, # 2-H, n.2-17JB). So, it would be possible to assume the existence of a third group of anthrax vaccines made from strains of Bacillus anthracis pXO1+/pXO2+. The feature that most distinguishes it from other vaccine strains is that despite a pattern plasmid identical to that of a pathogenic strain, it shows a residual pathogenicity that is really very low. The vaccine Carbosap was used in vaccination of cattle and sheep, although tests have shown the effectiveness of protection even in the case of caprine and in the equine. Efficacy trials of the vaccine have highlighted the high protective power which tends to set in after just one vaccination intervention. This particular property makes it very useful in common practice because its use is more advantageous compared to the Sterne which requires, on the contrary, two doses.

Link to Italian vaccines:

http://www.izsfg.it/izsportal/Base.aspx?frame=Attivita\CentriReferenza\Antrace\CERNAAttivit%C3%A0Ricerche.ascx&lang=IT.

Romania: Strain 1190R - Stamatin

This strain was developed by Nicolae Statmatin in 1937-38 at the Institute Pasteur in Bucharest. It is a non-capsulated strain (pX01+, pX02-). Details can be seen in Table 9. Information was mainly obtained from the 2 manufacturers that were identified:

Institute Pasteur Romania: **http://www.pasteur.ro/produse/antravac/**

Romvac: **http://www.romvac.ro/Blog%20Posts/carboromvac-2.html**

Please note that some information might have been "lost in translation".

Russia and Eastern/Central Europe: Strain 55-VNIIVViM and others

Strain 55 was developed by The National Research Institute for Veterinary Virology and Microbiology of Russia (VNIIVViM). Information about this vaccine can be seen in Table 9. The details have been obtained from internet searches, and pages containing information from what would look like official documents, and some manufacturers' information.

http://agrozoo.ru/text/vetprep_html/240.html

http://www.webvidal.ru/Veterinary/2004/LP_3170000.htm

http://www.alppp.ru/law/hozjajstvennaja-dejatelnost/selskoe-hozjajstvo/60/nastavlenie-po-primeneniyu-zhidkoj-vakciny-protiv-sibirskoj-jazvy-zhivotnyh-iz-shtamma-55.html

http://ruexport.org/eng/rus_export_catalog/17737/1221.html?printable

It seems that in order to overcome some of the issues of the strain 55, a new strain has been developed, the strain 363/11, obtained from an affected gilt. This is also a pX01+, pX02- strain. It looks like it has been patented in Russia: **http://russianpatents.com/patent/254/2544951.html**

There is also a patent for a combined vaccine against anthrax based on the binary combination of live spores vaccine strain STI-1 and protective antigen, obtained from microbial culture vaccine strain 55 VNIIVViM in order to create long-lasting immunity. **http://russianpatents.com/patent/222/2220742.html**.

Please note that some information might not be correct, due to the use of Google translate as the majority of the information is in Russian.

China

In China there are currently 3 vaccines. The Sterne vaccine, the Pasteur Type 2, and a PA oil emulsion vaccine (Sterne culture filtrate mixed with oil adjuvant) which was originally developed for goats.

Ukraine

There are some references about a live anthrax vaccine called K79-Z used in Ukraine, but information is very limited: **http://referatu.net.ua/referats/7569/148507**. It seems that the vaccine was developed at the Institute of Veterinary Medicine, National Academy of Agrarian Sciences of Ukraine:

http://ivm.kiev.ua/en/structure/scientific-departments/department-of-especially-dangerous-diseases/laboratory-of-anthrax-study.html. They also developed an experimental abacillar vaccine for anthrax called "Antrakol", in which the main component is exotoxin (obtained from *B. anthracis* strain K-79Z), which was successfully tested in experimental farms and has high performance:

http://www.stcu.int/documents/reports/distribution/tpf/ipf/bio/ua/pdf/Institute_Veter_Medicine.pdf

http://journals.nubip.edu.ua/index.php/Veterenarna/article/viewFile/5477/5390

Human vaccines

There are mainly 4 human vaccines. The ones produced in China, Russia, UK and USA.

UK and USA

In the UK and USA, human vaccines developed in the 1950s and 1960s respectively, are produced principally for military use. The UK vaccine is an alum-precipitated cell-free culture filtrate of the Sterne strain and it is called Anthrax Vaccine Precipitated (AVP). It was first introduced for works in at risk occupations in 1965, and licensed for human use in 1979 [38]. The vaccination plan is based on 3 primary doses of 0.5 ml IM at 3 week intervals, with booster at 32 weeks and thereafter annually. In the USA the vaccine is an aluminium hydroxide adsorbed cell-free culture filtrate of a non-capsulating, non-proteolytic derivative of bovine isolate V770 grown anaerobically in a fermenter, and is called Anthrax Vaccine Absorbed (AVA). The vaccination regime is 3 SC doses of 0.5 ml each, 2 weeks apart, followed by 3 additional SC injections at 6, 12 and 18 months, and then annual boosters. The AVA and the AVP vaccines made by batch culture of pX01+/pX02- *B. anthracis*, comprise predominantly PA with trace quantities of various other bacterial derived components. AVA contains traces of LF but is virtually free of EF, while AVP contains some LF and traces of EF. However, due to the method of production, the relative concentration of these proteins in consecutive batches can vary [11].

Very recently (23 Nov 2015), the US Food and Drug Administration approved a new indication for BioThrax (anthrax vaccine adsorbed) to prevent disease following suspected or confirmed exposure to *B. anthracis* (**http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm474027.htm**). The vaccine can be

used together with antibiotic treatment, to prevent disease after exposure to anthrax spores. Protective antibody levels, which were determined in rabbit and monkey studies, were used to predict efficacy in humans.

There is an ongoing effort to produce better defined vaccines for anthrax with contemporary formulations and presentations. This is based on an evolving understanding of the complex pathogenesis of anthrax infection and the fact that it is insufficient to merely reduce the bacteraemia with antibiotics, since beyond a certain tipping point, the toxaemia is fatal. To achieve a more defined vaccine, the recombinant expression of PA and/or LF has been pursued and a number of candidate rPA vaccines are currently in development and in clinical trials for safety. These have the advantage of being defined, highly purified formulations which are enriched for PA. Additionally, formulations comprising spore coat proteins in combination with PA have been proposed.

Non-clinical data from the use of these vaccines experimentally, would suggest that both AVA and AVP are efficacious in protecting animals, when given before and, within a defined time-frame, following exposure. In clinical use however, the AVA and AVP formulations can be reactogenic and current efforts are aimed at rationalizing the clinical dosing regimen to reduce dosing frequency whilst enhancing immunogenicity.

Russia

A live anthrax vaccine (LAV) for human use was developed in the former Soviet Union (FSU) by Russian microbiologists in the 1940s when acapsular variants (pX01+/pX02-) of *B. anthracis* were selected from the fully virulent strain Krasnaya Niva [39]. Initially the vaccine consisted of dry spores of two non-encapsulated *B. anthracis* strains, namely STI-1 (from Sanitary and Technical Institute) and NO 3, capable of producing the anthrax toxin complex. Subsequently, the latter strain was removed from the formulation, leaving STI-1 as the only component. According to multiple loci variable number tandem repeat analysis, STI-1 does not match any other genotypes including the vaccine strain Sterne, confirming its unique nature. Both cutaneous (scarification) and SC methods of vaccination are approved for human vaccination, and are associated with only mild reactions. Since the 1990s, a purified *B. anthracis* PA adsorbed on aluminium hydroxide was added to the vaccine, resulting in a combined anthrax vaccine formulation STI-1 +PA. Both vaccines demonstrated high efficacy against the 3 main clinical forms of anthrax, and were able to induce a robust antitoxic humoral immune response, as well as cell-mediated immunity in different animal models (mice, rabbits, guinea pigs, baboons). Moreover, vaccination with STI-1+PA accelerated immunity to anthrax, eliciting protection on days 7-10 after single injection of vaccine, while vaccination with STI-1 alone, required at least 2 sequential injections to achieve similar results. LAV was widely used for vaccination of livestock during the period 1947-1960s and was associated with a significant reduction in both human and animal cases on anthrax in several areas of the FSU (currently the strain 55 is the one used as a vaccine strain for cattle). Concerns remain about the efficacy and/or reactogenicity of current anthrax vaccines. A recombinant *B. anthracis* strain carrying the plasmid pUB110PA-1 that provided stable expression of the PA, has been described and was capable of protecting guinea pigs against experimental anthrax after a single immunization.

China

Lanzhou Institute of Biological products produces a live spore suspension of strain A16R in 50% glycerol and distilled water. It is used as a single dose by scarification in 2 spots on the skin with a single booster after 6 or 12 months, and annual boosters thereafter.

Table 9: Comparison of the different Anthrax livestock vaccines

	Sterne $34F_2$	Pasteur	Carbosap
Status	Most widely used anthrax vaccine in animals.	Abandoned in most countries. Still used in China.	Only used in Italy. Replaced by Sterne in 2006.
Type	Rough variant, toxigenic, non-capsulated. Has lost the pX02 plasmid that codes for capsule production (pX01+/pX02-)	Toxigenic capsulated strain, with reduced virulence for most species (pX01-, pX02+).	Toxigenic capsulated strain, with reduced virulence for most species (pX01+, pX02+).
Origin	Variant from a virulent bovine isolate in the 1930s, cultured on serum agar in elevated CO_2 atmosphere by Max Sterne at Onderstepoort (South Africa)	Obtained by Pasteur by cultivating strains at 42C.	Vaccine Institute of Asmara, during the II World War
Target species	All livestock and wildlife	Goats and horses.	Cattle and sheep
Other species	Care should be taken with goats and llamas. Horses slow at developing immunity.	There is a different formulation for cattle and sheep.	
Indications	Usually only one vaccination per year, preferably in spring. In high-risk zones, 2 doses are recommended (spring and autumn). Zebras: 2 initial doses, 8 weeks apart. Goats: 2 inoculations one month apart, being the first dose, 1/4 of the standard recommended dose.		
Immunity	Annual revaccination is usually recommended		After one dose. Shorter than the other vaccines.
Route	Usually SC. IM has been used for wildlife.	SC	SC
Dose & volume	OIE: Minimum 2-10 x10^6 culturable spores per dose for cattle, buffaloes and horses, and no less than 1-5x10^6 culturable spores per dose for sheep, goats and pigs. Volumes are usually 2 ml for cattle and horses, 1 ml for small ruminants and for foals, calves and small animals 0.5 ml.	Goats & horses: 0.125 ml Cattle >6 months: 0.25 ml Cattle <6 months, sheep: 0.125 ml	Cattle: 0.250 ml Sheep: 0.125 ml
Serology on standard tests	Great variability in titres among animals with similar vaccination records.		
Withdrawal period	42 days for meat (WHO recommendation), nil per milk.		
Pathogenicity	Residual pathogenicity for goats and llamas		Low
Efficacy	Protection tends to be less than 100% if they have only received a single dose, and the dose was less than 10^7 spores.		
Zoonotic characteristics	Potentially infectious for humans. Self-injection by the operator can lead to infection, but few, if any, serious events have ever been recorded.		
Use in pregnant animals	No records of adverse events related to pregnancies.		
Other side effects		3% mortality risk.	
First used	1939		Italy: 1949
Large scale use	Yes	Used in the past, and in Italy until 2006. Now only in China, but not sure at which extent	It was used in Italy until 2006.
Others		Originally they were 2 types: I and II. They were poorly standardised, some residual virulence or some inactive.	

Table continued: Comparison of the different Anthrax vaccines

	Strain 55	1190R
Status	Used in Central and Eastern Europe	Romania
Type	Toxigenic, non-capsulating strain, analogous to Sterne	Stamatin strain, non-capsulated (pX01+/pX02-), similar to Sterne
Origin	strain VNIIVViM 55	Developed by Nicolae Statmatin in 1937-38, Pasteur Institute Bucharest (Romania)
Target species	All farm animals	Cattle, sheep, goats, pigs and horses
Other species		
Indications	Do not vaccinate animals younger than 3 months.	Vaccinate in the spring, 14 days before moving grazing animals.
Immunity	Immunity develops after 10 days and last 12 months	Immunity starts 21 days after vaccination, and lasts 12 months
Route	SC or ID	SC
Dose & volume	Doses vary with different versions of the vaccine. One manuf: Sheep and goats: 0.5 ml in the middle third of the neck, in the hairless inner thigh or chest. Horses, cattle, deer, camels, donkeys, and fur-bearing animals: 1 ml in the middle third of the neck, pigs: 1 ml in the inner thigh or behind the ear.	Antravac (Inst Pasteur Romania) has a concentration of 2.5 -5.5 x10^7 UFC/ml. The recommended dose is for cattle 0.5 ml, for sheep 0.2 and goats 0.1 ml (in the tail crease), for pigs and horses 0.2 ml. Vaccine to be used within 8 hours of opening.
Serology on standard tests	Low antigenicity. Titers 2-8 times lower than to Sterne strain.	
Withdrawal period	Meat: 2 weeks, milk: nil.	Both manufacturers found recommend zero days for meat and milk.
Pathogenicity		
Efficacy	Protects 40-100% of population from infection with virulent field isolates.	
Zoonotic characteristics		Potentially infectious for humans. Self-inoculation requires urgent medical advice and supervision.
Use in pregnant animals	Do not use in the last month of pregnancy	Mechanical abortions might occur if animals vaccinated during last month of pregnancy. Do not use 3 weeks after giving birth.
Other side effects	Possible increase in body temperature, local reactions at the injection site.	Light oedemas may occur at the side of injection, and sometimes an insignificant decrease in milk. Anaphylactic reactions 2-3% animals
First used		1937
Large scale use	Central and Eastern Europe	Romania
Others	Not a wide protective range against field isolates circulating in Russia and residual reactogenicity.	

Main vaccine needs:

There is a need for a vaccine that:

- Is not affected by antibiotic treatment, has a stronger initial immunity and can be used at the onset of an outbreak.
- Can be given orally (especially for wildlife)
- Safer for humans
- Does not have withdrawal period
- A longer duration of immunity would also be desirable (especially for wildlife).
- vaccines

Commercial vaccines manufactured in Africa and Asia

The information summarised in Tables 10 and 11 below, is based on information from The Center for Food Security and Public health, Iowa State University (**www.cfsph.iastate.edu/vaccines/index.php** and Vetvac (**www.vetvac.org**). More details have not been gathered, as another consultant has been commissioned to perform this task.

Table 10: Manufacturers of Anthrax vaccines in Africa and Asia.

Manufacturer	Country	Name & Strain	Vaccine Type	Countries distribution
AFRICA				
Botswana Vaccine Institute	Botswana	CABOVAX C® Strain: Sterne	Live	Botswana
National Veterinary Research Institute	Ethiopia	Anthrax Spore Vaccine	Live	Ethiopia
National Veterinary Research Institute	Mozambique			Mozambique
National Veterinary Research Institute – Vom	Nigeria	Anthrax Spore Vaccine (ASV) Sterne (34F2)	Live	Nigeria

Onderstepoort Biological Products	South Africa	Anthrax Spore Vaccine Sterne (34F2)	Live	South Africa
MSD Animal Health (Merck)	South Africa	BLANTHRAX (Blackleg, Anthrax) Sterne 34F2 (A)	Live	South Africa
	South Africa	BOTUTHRAX (Botulism, Anthrax) Types C, D (B) Sterne 34F2 (A)	Killed (B), Live (A)	South Africa
	South Africa	SUPAVAX (Anthrax, Botulism, Blackleg) Types C1+2, D (B) Sterne 34F2 (A)	Killed (B), Live (A)	South Africa
National Veterinary Research Institute	Sudan	Anthrax live Sterne F34	Live	Sudan
Tanzania Veterinary Laboratory Agency	Tanzania		Live	Tanzania
Central Veterinary Research Institute	Zambia			
ASIA				
Tiankang Biopharmacuetical	China	No. II Anthrax Vaccine		China
		No Capsule Anthrax Vaccine		China
Indian Immunologicals Limited	India	Raksha Anthrax Strain: non-capsulated	Live	India
Institute of Animal Health and Veterinary Biologicals	India	Anthrax Spore (IP) Strain: Weybridge	Live	India
Institute of Veterinary Preventive Medicine	India	Anthrax Spore Vaccine Strain: Sterne (34F2)	Live	India

Jordan Bio-Industries Center (JOVAC)	Jordan	ANTHRAVAC Strain: Sterne (34F2)	Live	Ethiopia and others
KAKETSUKEN (Chemo-Sero-Therapeutic Research Institute)	Japan	Anthrax Vaccine Strain: N/A	Live	Japan
Pt. Vaksindo Satwa Nusantara	Indonesia	VAKSIMUNE Sterne (34F2)		Indonesia, Myanmar, Nigeria, Vietnam and others
Pusvetma	Indonesia	Anthravet		Indonesia
Philippines Bureau of Animal Industry	Philippines	Anthrax Vaccine	N/A	Philippines
CAVAC (ChoongAng Vaccine Laboratories Co., Ltd.)	South Korea	BoviShot® Anth-Leg (Anthrax, Blackleg Disease) Strain: N/A	Live	South Korea
Green Cross Veterinary Products	South Korea	Anthrax-Blackleg combined vaccine	A: Live B: Killed	South Korea

Commercial vaccines imported into Africa and Asia

The information in Table 11 is based on the questionnaires sent to the Directors of Veterinary Services office and regulators of the countries of interest. Note that some vaccines might have been imported under DVS dispensation, and they are not necessary licensed in the country.

Table 11: Manufacturers of Anthrax vaccines in Africa and Asia.

Country	Vaccine name	Strain or type	Country of origin	Doses imported 2015	Doses imported 2014	Doses imported 2013	Doses imported 2012
ASIA							
Bangladesh	-	-	-	-	-	-	-
India							
Indonesia							
Myanmar (Burma)	-	-	-	-	-	-	-
Nepal	-	-	-	-	-	-	-
Vietnam	-	-	-	-	-	-	-
AFRICA							
Burkina Faso							
Côte d'Ivoire (Ivory Coast)	Anthrax (ND)	*Bacillus anthracis*	Ethiopia	200,000	400,000		
Ethiopia			Jordan				
Kenya	-	-	-	-	-	-	-
Madagascar							
Malawi	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mali	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Mozambique							
Rwanda	Bivax		Coopers/Kenya	360,000	220,000	160,000	120,000
Senegal							
South Africa							
Tanzania *	Bacillus Anthrax	34F2 Spore	South Africa	4	-	-	-
Uganda	-	-	-	-	-	-	-
Zambia	-	34F2	South Africa	2,000	2,000	28,700	-
	-	34F2	Uruguay	97,800	40,000		

- Questionnaire received, no information provided (blank).
* Information supplied by the local Regulatory Authorities

Other comments

JOVAC, the manufacturer from Jordan was also sent a questionnaire designed for key importers into the region. They confirmed that they export *B. anthracis* vaccine (Anthravac), strain Sterne 34F2 to Asia and Africa. They did not specify the countries or the volumes.

According to CFSPH, Laboratorio Prondil from Uruguay, produces Anthrax vaccine alone and in combination, and the vaccines are licenced in Kenya, Morocco, South Africa, Zambia, Zimbabwe and other countries.

Combination vaccines

Current use: Anthrax is a vaccine that is already used in combination vaccines by some laboratories. It is usually combined with Blackleg disease (*Clostridium chauvoei)* and/or with Botulism (*Clostridium botulinum* types C and D).

Desirable combinations: They might vary depending on the area, and the key diseases present. There is not an obvious “universal” combination that would be desired.

Characteristics of Ideal Vaccine Candidates for Smallholders

The Target Product Profiles (TPPs) reflect the availability and utility of current agents and incorporate features that will be necessary to improve on the current products and to address unmet needs, taking into account the particular requirements of the poorest livestock keepers.

The TPPs are more robust when they include the opinions and consider the needs of the different stakeholders. While efforts have been made to encompass them, the TPP showed in Table 12 below, should be considered a proposal, a live document subject to improvements.

Information on current vaccines has been obtained from the datasheet of different products of the most common vaccine used globally, the Sterne 34F2. The ones reviewed are:

Colorado Serum Company: **http://www.colorado-serum.com/csc/anthrax.html**

OBP: **http://www.obpvaccines.co.za/Cms_Data/Contents/OBPDB/Folders/Product/~contents/9BG2R9F8KN3KQFGC/1015%20Anthrax%20Spore_PI.pdf**

BVI: **http://www.bvi.co.bw/common_up/bvi-new/files/CARBOVAX-C.pdf**

Jovac: **http://www.jovaccenter.com/userfiles/file/9-06-2014/Large%20Animals%20Vaccines%20/Anthrax-En.pdf**

Syva: **http://www.syva.es/pdfs/p_2015-06-30_1435653056.pdf**

Table 12: Manufacturers of Anthrax vaccines in Africa and Asia.

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Immunogen with protective antigens for *Bacillus anthracis*	Immunogen with protective antigens for *Bacillus anthracis*
2	Indication for use	For active immunization of cattle, sheep, goats, pigs, horses, mules, donkeys and camels. Revaccinate annually approx. 4 weeks prior to the time the disease usually appears.	For active immunization of cattle, water buffalo, sheep, goats, pigs, horses, mules, donkeys, camels and wildlife.
3	Recommended species	Cattle, sheep, goats, pigs, horses, mules, donkeys and camels.	Cattle, buffalo, sheep, goats and pigs. Also all susceptible animals, including susceptible wildlife.
4	Recommended dose	0.5 - 1 -2 ml SC (some manufacturers have different recommendations and doses for different species)	Same dose for all species (1 or 2 ml)
5	Pharmaceutical form	Injectable suspension	Ready to use solution/suspension
6	Route of administration	SC or IM (some manufacturers mention ID) Goats must be injected in the inner thigh or tailfold as these animals are inclined to show allergic reactions with swellings at the site of injection, which may cause suffocation if the inoculation is given in the neck.	SC, Intramuscular or oral (bait vaccine, this last route, very important for wildlife)
7	Regimen - primary vaccination	Two vaccines, 2-3 weeks apart in heavy contaminated areas.	Single lifetime dose
8	Regimen - booster	Revaccinate annually.	Lifelong immunity after primary vaccination

9	Epidemiological relevance	Protection against *B. anthracis*	Protection against *B. anthracis* and all possible mutants
10	Recommended age at first vaccination	From 3 months of age	From 1-2 months of age, when other vaccines are applied.
11	Onset of immunity	7-10 days	<7 days (very important for outbreaks)
12	Duration of immunity	1 year	Lifelong immunity
13	Expected efficacy	To prevent disease & prevent mortality.	To prevent infection and transmission in 100% of the animals. No disease & no mortality in vaccinated animals after virulent challenge.
14	Expected safety	Possible swelling at the site of injection, which subsides after a few days. Adverse reactions have been reported in young and miniature horses. Anaphylactoid reaction may occur.	No post-vaccinal reactions at any age. Safe for pregnant animals at any stage. Safe for all sexes at any age.
15	Withdrawal period	Meat: 0 - 14 - 42 days (depending on manufacturer) – WHO recommends 42 days.	Nil for milk and meat
16	Special requirements for animals	Do not vaccinate un-healthy animals. All working animals should be rested for 7-10 days after vaccination. Avoid antibiotic therapy 7 days before and 14 days after vaccination.	Vaccinate all animals
17	Special requirements for persons		None
18	Package size	10 -100 doses	Multiple pack size from 5 doses
19	Price to end user		

20	Storage condition and shelf-life as packaged for sale	Stable at 4-8°C for 24 months	Stable at 30°C for 24 months
21	In-use stability		24 hours or greater
22	Other: Interference with antibiotics	Antibiotic treatment will interfere with vaccine efficacy.	Antibiotic treatment will not interfere with vaccine efficacy.

Limitations

Scientific quality: The publications and data from the different research groups, should be carefully evaluated. The use of good science and good experimental design with use of proper controls, adequate numbers, suitable challenge model, reproduction of results by them and by independent groups, and appropriate analysis has not been verified for this monograph. If any of these projects were to be pursued, a detailed peer review taking into account the above considerations is strongly recommended.

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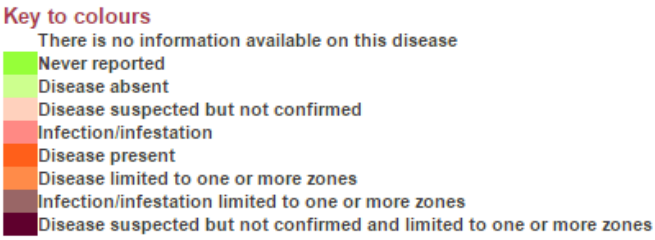
ANNEX 1: Additional data on disease presence and incidence

Reports to OIE on Anthrax:



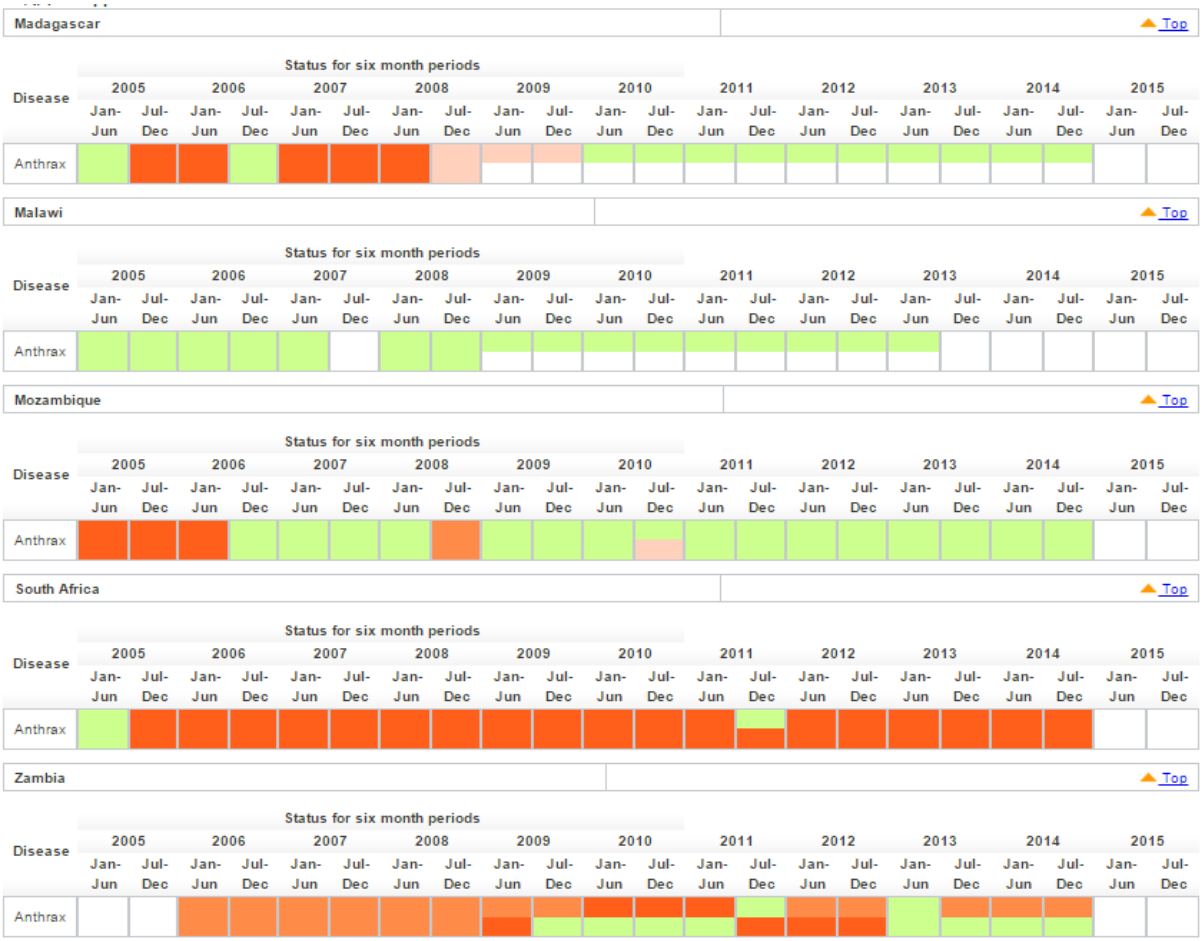
When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

Anthrax in Asia: Bangladesh, India, Indonesia, Myanmar, Nepal and Vietnam

Anthrax in Western Africa: Burkina Faso, Ivory Coast, Mali and Senegal

Anthrax in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda

Anthrax in Southern Africa: Madagascar, Malawi, Mozambique, South Africa and Zambia



ANNEX 2: Additional research results from Mr Ndumnego (Dr Beyer group)

Using a passive mouse protection model as substitute for direct lethal anthrax challenge in target animals

OC Ndumnego[1], S Koehler[2], F. Buyuk[3], O. Celebi[3], S. Otlu[3], M. Doganay[4], M. Sahin[3], J Crafford[1], W Beyer[2], H van Heerden[1]

[1]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, e-mail: okeyndumnego@yahoo.com, [2]Department of Environmental and Animal Hygiene, Institute for Animal Sciences, University of Hohenheim, Stuttgart, Germany, [3]Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey, [4]Department of Infectious Diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey

Rationale for the study

- The development and testing of new veterinary vaccines in target animals are largely encumbered by ethical and regulatory concerns.
- High level bio-containment facilities are required for vaccine trials against anthrax.
- Level 3 biosafety facilities are expensive to build and maintain, more so, scarce in resource-poor countries where anthrax is endemic.
- Few data exist on alternative, safer methods for evaluating new vaccine candidates that correlates well with protection in target animals.
- An *in vivo* mouse model was evaluated and compared to a susceptible host (goat) challenge trial in order to assess the protectivity of non-living vaccine antigens.

Approach

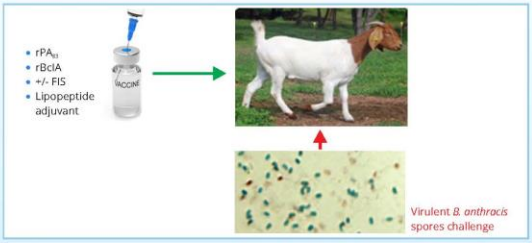


Figure 1. Direct virulent *Bacillus anthracis* spores challenge in goats following vaccination with non-living vaccine antigens. The three vaccine groups comprised of rPA+rBclA+lipopeptide adjuvant (n = 8), rPA+rBclA+FIS+lipopeptide adjuvant (n = 10) and unvaccinated controls (n = 4). Vaccine candidates were administered thrice at 3 weeks intervals and lethal challenge with ~1000 virulent *B. anthracis* spores 5 weeks later. Goats were monitored for 15 days following lethal challenge.

Figure 2. Lethal challenge of A/J mice with Sterne 34F2 spores following in vivo transfer of caprine immune sera. The three caprine vaccine groups comprised of rPA+rBclA+lipopeptide adjuvant, rPA+rBclA+FIS+lipopeptide adjuvant and unvaccinated controls. Vaccine candidates were administered thrice at 3 weeks intervals and serum collected 4 weeks after the last vaccination. Sera from the various vaccine groups were transferred (i.p) to groups of A/J mice and lethal challenge performed with 1.92×10^5 Sterne 34F2 vaccine spores. The mice were monitored for 14 days following lethal challenge.

Results

- Goats vaccinated with rPA, rBclA and FIS generated the highest antibody and toxin neutralization titres (Fig. 3 and 4).
- Survival was highest in the rPA+rBclA+FIS group (80%) with rPA+rBclA having 50% survival following direct lethal challenge (Fig 5).
- *In vivo* transfer of immune sera from rPA+rBclA+FIS vaccinated goats protected 73% of naïve A/J mice against lethal challenge.
- rPA+rBclA vaccine candidates alone protected 68% of challenged mice.

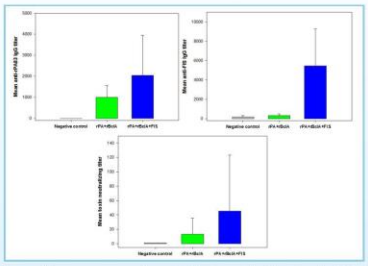


Figure 3. Mean humoral immune responses in goats following vaccination at weeks 0, 3 and 6 with non-living *Bacillus anthracis* antigens. Serum was collected and analysed five weeks after the last vaccination

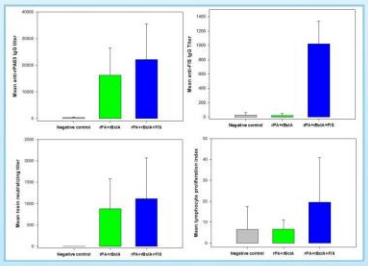


Figure 4. Mean humoral immune responses and lymphocyte proliferation in goats following vaccination at weeks 0, 3 and 6 with non-living *Bacillus anthracis* antigens. Serum was collected, analysed and used for in vivo mouse protection test four weeks after the last vaccination.

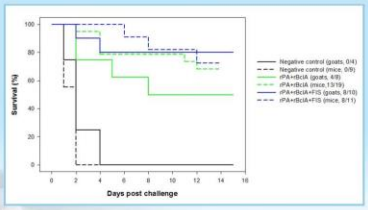
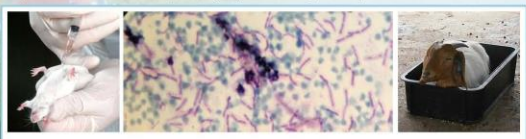


Figure 5. Survival data of animals either immunized with non-living *Bacillus anthracis* antigens (goats) or inoculated with immune sera from goats vaccinated with similar antigens (A/J mice). Lethal challenge was performed with either a fully virulent wild type *B. anthracis* strain in the goats or Sterne 34F2 vaccine strain (A/J mice). Survival was monitored for up to 15 days in goats and 14 days in the A/J mice.



Conclusion

The passive mouse protection assay can serve as a viable alternative to large target animal challenge trials. This model has lower biosafety requirements, cheaper and cost effective especially in resource-poor settings.

Faculty of Veterinary Science
National Research Foundation
Deutsche Forschungsgemeinschaft

Abstract:

Comparing immunogenicity of non-living anthrax vaccine candidates in combination with simultaneous antibiotic treatment in goats

OC Ndumnego[1], S Koehler[2], J Crafford[1], W Beyer[2], H van Heerden[1]

[1]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa;

[2] Institute for Animal Sciences, Dept. of Environmental and Animal Hygiene, University of Hohenheim, Stuttgart, Germany.

Potential advantages of non-living anthrax vaccines include the simultaneous use of antibiotics and vaccination for the treatment of valuable livestock and endangered wildlife during anthrax outbreaks. In this study we assessed the immunogenicity of these vaccine antigens, either in combination with or without antibiotics, in goats compared to the live spore Sterne vaccine.

The vaccine antigens comprised of recombinant protective antigen (rPA), spore-specific bacillus collagen-like antigen (rBclA) and formaldehyde inactivated spores (FIS). Groups of age-matched goats (n = 5) were vaccinated 3 times at three weeks interval. Two control groups were vaccinated twice with the Sterne live spore vaccine with or without Penicillin G (Pen G). Serum and peripheral blood mononuclear cells (PBMC) were isolated before every vaccination and specific antibody responses to homologous antigens measured using ELISA, toxin neutralisation assay (TNA) and the lymphocyte proliferation assay.

Results showed goats receiving the full antigen complement (rPA + rBclA + FIS) had the highest antibody and TNA titres. Simultaneous administration of these antigens with Pen G had no diminishing effect on the immune response. Furthermore, comparing the immunogenicity between these groups (Pen G treated or untreated) and the twice-Sterne vaccinated goats revealed equivalent development of titres, after the second vaccination. The control group vaccinated with Sterne vaccine and simultaneously treated with Pen G showed no antibody titres at any time-point. In conclusion, the current data indicate promising potential for further development of non-living anthrax vaccines in ruminants which can be used as an alternative to the Sterne spore live vaccine under circumstances which call for antibiotic treatment and vaccination.